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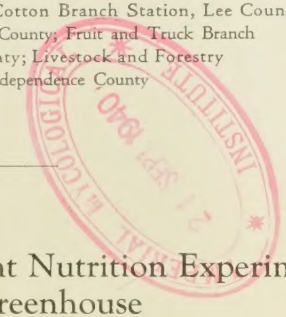

Factorial Design in Plant Nutrition Experiments in the Greenhouse

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FACTORIAL DESIGN IN PLANT NUTRITION EXPERIMENTS IN THE GREENHOUSE

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Experimental work with plants is usually designed so that all variable modifying factors, except the one to be studied, are held as constant or as uniform as possible in order to obtain precise information concerning the effect of the one factor. It follows that a given level of each of the other variable factors must be arbitrarily chosen and that the observed results from the factor being studied may be interpreted only for this specific combination of the extrinsic factors. A different combination of extraneous factors might well change the results from the one being studied. That is, the entire experiment may be delimited by a special set of conditions that preclude anything but a narrow interpretation of the results.

Research work with plants necessarily has, as its ultimate goal, a better understanding of plant responses as they occur under natural conditions. Under these conditions, the whole gamut of influential factors may vary simultaneously. Therefore, as many variable factors as possible should be studied concurrently so as not only to observe the plant response to an individual factor, but also to study the effect of combinations of factors upon plant response. Interactions between factors may reveal as much information as the main effect of the factors themselves. The response of a crop to a fertilizer may depend upon the variety used, making it desirable to use several varieties, and thereby obtain information on a possible variety-fertilizer treatment interaction.

Factorial design permits the investigator to study the importance of interactions between factors as well as the effects of the factors themselves. The basic requirement is that each level of each factor will occur in combination with each level of every other factor being studied. In addition to allowing for measurement of interactions, factorial design is highly efficient, because, through repeated restratification of the data, each main effect is measured with the use of all the observations. Thus, replicates

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need often be but few, and, as shown by Brandt *(1)*, reliable information can be obtained without the use of any true replications. In fact, it is often found necessary to limit replications in order to accommodate the desired number of factors at adequate levels, since the addition of each factor to the design multiplies the plot number by the number of levels taken within each given factor. Consequently the experimenter should plan with full recognition of the rate of progression for additive factors in order to keep his number of plots within the limits of available experimental space. Caution should also be exercised in studies involving factors for which the range of effective variability is unknown, because when a factor is studied at levels outside the effective range, then no interaction with other factors could occur and much work is needlessly done.

When it is known or strongly suspected that all factors to be studied may be found to have interactive effects, then factorial design will probably be found the most efficient means of obtaining all desired information. If, on the other hand, no interaction is suspected it might be preferable to employ some other type of design—selecting the one most adaptable to the problem. Often the available space, particularly in the greenhouse, may limit possibilities and be a determining factor in the selection. In any case, the design, factorial or otherwise, should be selected, and at times modified, so that it offers the best approach to a study of the problem, and facilitates the proper statistical evaluation of the resultant data. While the design of the experiment essentially dictates the type of analysis, it is possible to utilize modifications to meet certain contingencies without altering the accuracy of the statistical measurements. In fact certain conditions may develop during the course of an experiment that necessitates some modification in the procedure of analysis which was not indicated by the nature of the design. Furthermore, certain experiments may be unfortunately designed—through either mistakes or misconceptions, but if they are otherwise successful there is often a modification of routine analysis that may allow complete salvage of the resultant information.

In the course of a series of investigations dealing with the nutrition of the cotton plant the writers felt that, for the reasons just discussed, the precepts of factorial design should be incorporated into many of their experiments especially since preliminary, exploratory studies had indicated interactive effects among many of the factors to be investigated. As these experiments progressed the authors' experience with the more recent designs became con-

siderably augmented, and they became more cognizant of the problems that were involved in both the selection of proper design and method of subsequent analysis of the resultant data. Thus, in some of the examples selected for presentation, there are certain unorthodox features of experimental design. Some of these were occasioned by peculiarities of the experimental conditions and were, perhaps, unavoidable, yet other features might have been changed to give a more conventional design were the experiments to be repeated. Not only do these experiments present certain pitfalls that should be avoided, but illustrate how data from experiments of unconventional design can be evaluated statistically without undue loss of precision in the measurements. Conversely, experimental examples are discussed which, though slightly unorthodox in design, offer the best possible approach to a compromise between the theoretically ideal and the practical limitations of certain working or experimental conditions.

The examples here discussed, and conditions of limitations emphasized, pertain particularly to plant nutrition studies in the greenhouse. However, many of these greenhouse designs are applicable to similar studies under field conditions although the sources for upset might be found quite different. While this discussion is limited to a study of certain designs and analyses that were peculiar to specific conditions of experimentation, it is hoped that the presentation may be of interest to other investigators faced with similar or related problems. It is not within the scope of this bulletin to go into basic mathematics of this method inasmuch as this phase of the work has been amply covered by Yates and Cochran in England and Brandt in the United States. It is also believed that the presentation of the material should avoid fundamental technicalities, since it is not written for specialists in statistics; consequently, only those phases of statistical interpretation that will make for clarity of the entire presentation are presented.

The four experiments that are described and discussed in this bulletin were carried out in the greenhouses of the Arkansas Agricultural Experiment Station during the summer of 1938. Each experiment will be introduced to present a different phase of factorial design together with a suitable method of analysis of the results.

Experiment A presents a $3 \times 3 \times 2 \times 2$ systematized split-plot design with the block arrangement disrupted by peculiarities in bench space. Experiment B describes a $3 \times 3 \times 3 \times 2 \times 2$ design with the two degrees of freedom of each three-level factor

partitioned into individual degrees of freedom. Experiment C presents the confounding of a higher order interaction with subblocks in a $2 \times 2 \times 2 \times 2$ design. Experiment D indicates the complications introduced by a three-level factor when confounding a $3 \times 2 \times 2 \times 2$ design with subblocks.

Since the purpose of this bulletin is to present certain statistical procedures that may be useful to investigators conducting plant research, it appears unwise to unnecessarily complicate the main thesis by the inclusion of biological or agricultural interpretations of any data used except to exemplify the methods. A study of the biological applications of the data will appear in subsequent publications.

The three varieties of cotton used in these experiments are Cook 307, Rowden 2088, and Half & Half.

EXPERIMENT A

Experiment A is concerned with the effect of nitrogen, phosphorus, and potassium nutrition upon the susceptibility of three varieties of cotton, when grown in the greenhouse in sand nutrient culture, to fusarium wilt *(8)*. Nitrogen was supplied at three levels, and phosphorus and potassium at two levels. There were four replicates, or series, in the experiment, each replicate comprising a block of all combinations of treatments and varieties. Records of wilt intensity in each culture were taken periodically. A coded value *(9)*, involving time and intensity of disease, was used in evaluating the "wilt" status of the plants in each culture at the end of the experiment. These coded values are used to illustrate the analysis of the experiment.

The arrangement of the pots on the three benches available in the greenhouse for this experiment is presented in Figure 1. The arrangement was wholly systematized. Each three pots in a tier across the bench received identical nutrient treatment. Each tier consisted of a pot of plants of each variety systematically arranged as to position in the tier. The treatments occurred in the identical uniform order in each block. By referring to Figure 1, in which the composition of each treatment is keyed to a given number, it will be seen that the levels of nitrogen supply correspond to subblocks within the main blocks. Also, levels of phosphorus supply form "split-plots" within the nitrogen subblocks; and levels of potassium supply similarly subdivide the phosphorus "split-plots," with varieties as the ultimate "split-plots." The blocks are not so constituted as to facilitate full

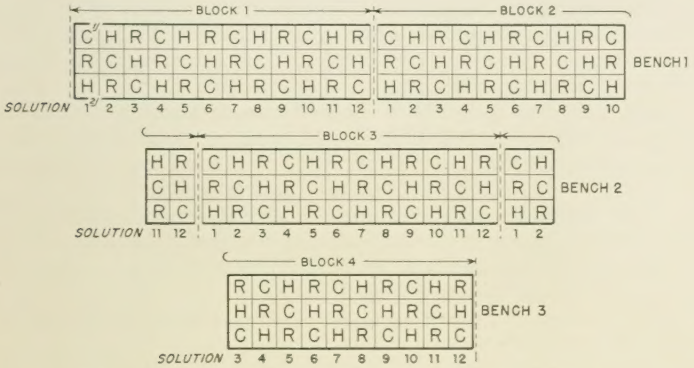

Figure 1. ARRANGEMENT OF CULTURES IN EXPERIMENT A.

[1] Key to varieties (each letter designates one jar):
C = Cook 307
R = Rowden 2088
H = Half & Half

[2] Key to treatments:
1 = Low N, low P, low K
2 = Low N, low P, high K
3 = Low N, high P, low K
4 = Low N, high P, high K
5 = Medium N, low P, low K
6 = Medium N, low P, high K
7 = Medium N, high P, low K
8 = Medium N, high P, high K
9 = High N, low P, low K
10 = High N, low P, high K
11 = High N, high P, low K
12 = High N, high P, high K

statistical interpretation, inasmuch as that portion of a given block which is not included on one bench, because of lack of space, is carried over to the next available bench—an admittedly unadvisable procedure which might have been prevented if the writers were, at that time, sufficiently experienced with the newer practices for reducing block size.

A study of this design reveals that it is subject to other criticism from the statistical point of view. But it does present several practical problems that arise in plant nutrition work in the greenhouse, since it is not always possible to obtain a perfect experimental design on greenhouse benches, and the experimenter may be forced to use that which is feasible and suited to the study in mind. As in the present case, the greenhouse benches were nonuniform in dimensions, and the distribution of the space available for the experiment precluded the use of a design that would include an orthodox arrangement of the blocks. Undoubtedly, one of the most serious criticisms of arrangements similar to the one used in Experiment A is the derangement of the component blocks, thereby removing the possibility of satis-

factorily taking into account the significance of block differences. While the information on location effects is not lost, it is obscured. If possible a given block should be entirely confined to a continuous portion of a single bench.

One of the orthodox principles of experimental design is that the treatment combinations assigned to plots within a block shall be made in a randomized manner *(4)*. The complete systematization of this experiment, and the fact that treatment levels correspond with split-plots, are contrary to the concepts of experimental design necessary for the use of the ordinary, or unmodified, analysis of variance with a single fully pooled error. However, certain conditions prevail in greenhouse work which may have sufficient weight to merit a modification of the orthodox design used in field plots. For example, nutrient solutions were applied to these pots twice daily; and the fact that all three pots in a tier received the same nutrient solution greatly contributed to the elimination of errors in nutrient applications. Previous experience had shown that when pots of the various treatments were fully randomized, within main blocks, it was nearly impossible to prevent the occasional application of the wrong nutrient to a given jar. In some cases, such an error completely obliterated the significance of the treatment, thereby contributing greater error to the experiment than the dubious statistical error arising from applying uniform nutrient treatment to the three jars in a tier.

The fact that treatment levels are confounded with split-plots may militate against the ultimate desiderata of experimental design. If properly analyzed, however, this arrangement can be considered quite satisfactory, and, in some instances, may provide certain advantages. Thus, in Experiment A, all plants receiving a high level of nitrogen supply were confined to distinct subblocks, as were those receiving intermediate and low supplies of N; *e. g.*, tiers one through 4 formed a low nitrogen subblock, tiers 5 through 8 formed a medium nitrogen subblock; and tiers 9 through 12 formed a high nitrogen subblock. Plants receiving a high supply of nitrogen are usually larger and more vigorous than those receiving a low N supply. Consequently, plants that are small, because of nutritional conditions, may have their metabolic status modified depending upon whether they are being grown among other small plants or among large plants that partially shade them. This latter condition was avoided by the split-plot design. Unfortunately, though, the split-plots were not

randomized within the blocks: Plants of a given treatment in the various blocks were always in a juxtaposition to plants uniformly treated in all blocks. It is reasonable to assume that such a contingency may have introduced a bias into the experiment, a situation which should be avoided. The foregoing discussion is not intended to convey the idea that there is anything theoretically wrong with split-plot designs. On the contrary, as in the above case, it may be an exceedingly useful design for certain purposes. It is only necessary to caution that it be properly utilized and almost obligatory that the analysis be modified to conform with the subdivisions for comparisons that are dictated by the nature of the design.

The systematization of varieties within the treatments should not, in this experiment, be considered a serious defect. Preliminary experiments of similar nature had demonstrated that, although there were effects of both temperature and light gradients through large areas of the greenhouse, any small area, such as one tier of three pots, was free from significant locational effects. And, as will be seen in the analysis of these data, block effects are not significant, thus militating against the chance that there will be any appreciable locational effects within the smaller areas comprising the three-jar tiers across the bench. Even if slight differences did result from position (perhaps in outside rows), it would be far from serious because, in this study, the main effects for varieties are nearly beyond any need of statistical substantiation. Furthermore, Love and Craig *(6)* have indicated that in field trials the systematization of varieties does not seriously alter the accuracy of measurement, as compared to similar trials where varieties are randomized. Even though randomization may have been preferable, there remains little basis for the contention that lack of varietal randomization has detracted from the result.

Analysis of Experiment A

The data of Experiment A, consisting of disease code values for individual cultures of plants are presented in Table 1. A more complete comprehension of the implications within this data may be gained from the summaries in Table 2. If one were only interested in the obvious effects evident in Table 2, it would be unnecessary to carry out an involved statistical analysis. For example, the differences between the mean values for the levels within each main factor are of sufficient magnitude to indicate that all main effects are making a significant contribution to variability within the experiment. Since all responses of main

TABLE 1. DISEASE CODE VALUES OF PLANTS IN INDIVIDUAL POTS AT TERMINATION OF EXPERIMENT

Solution No.	Block I			Block II			Block III			Block IV		
	C[1]	R[1]	H[1]	C	R	H	C	R	H	C	R	H
1	6.0	1.1	9.5	2.1	0.0	3.7	0.0	4.1	8.0	0.0	2.5	5.0
2	.0	9.1	24.6	.0	.0	3.2	.0	6.1	.0	.0	.0	.0
3	.9	.0	25.7	.0	3.0	12.2	.0	12.0	8.2	3.0	.0	33.8
4	.0	.0	25.5	6.5	2.2	8.3	.0	.0	33.6	.0	2.2	9.9
5	.0	7.9	9.6	.0	.0	7.9	.0	.0	7.5	.0	.0	24.5
6	.0	.0	23.4	.0	.0	16.4	8.0	6.7	3.5	1.5	.0	1.9
7	1.9	14.4	31.1	4.1	4.4	34.0	5.6	8.4	32.6	1.4	5.0	51.1
8	.0	4.6	19.9	.0	.0	34.7	.8	.4	11.6	.4	5.0	31.9
9	7.2	11.3	40.2	.0	1.2	6.7	7.2	.0	42.3	.0	7.4	9.7
10	.0	2.6	8.2	.0	2.2	.0	.0	3.3	22.2	.0	3.0	38.1
11	26.2	5.2	47.7	10.8	15.9	78.1	2.2	16.0	55.5	7.9	11.4	39.2
12	.0	13.0	42.7	1.5	9.4	22.1	.0	14.4	37.1	2.4	12.7	10.1
Block totals		419.5			290.6			357.3			321.0	

[1] C = Cook 307; R = Rowden 2088; H = Half & Half.

effects were of the order of 50 per cent or greater and were fairly uniform for respective blocks (Table 1), it is reasonable to infer that analysis would merely confirm these deductions based on inspection. However, the importance of interrelationships of the contributing factors is not manifest even for the first order interactions presented as summaries in Table 2. As the main advantage of a factorially designed experiment lies in the facilities for studying interrelationships of factors, much valuable informa-

TABLE 2. MEANS OF DISEASE CODE VALUES FOR ALL MAIN EFFECTS AND FIRST ORDER INTERACTIONS

Variety	Low N	Medium N	High N	Low P	High P	Low K	High K	Mean
Cook 307	1.16	1.48	4.09	1.35	3.15	3.60	0.88	2.24
Rowden 2088	2.64	3.55	8.06	2.85	6.65	5.47	4.04	4.75
Half & Half	13.20	21.35	31.24	13.17	30.69	25.99	17.87	21.93
Mean	5.67	8.79	14.46	5.79	13.50	11.69	7.60	

N level	Low P	High P	Low K	High K
Low N	3.54	7.79	5.87	5.47
Medium N	4.95	12.64	10.48	7.11
High N	8.87	20.06	18.72	10.20
Mean	5.78	13.50	11.69	7.59

P level	Low K	High K
Low P	6.46	5.11
High P	16.91	10.08
Mean	11.69	7.60

tion might be lost if these interactive effects were not tested statistically.

Prior to presenting an analysis of these data, it should be emphasized that it embodies considerable variability, with a range in observations from 0.0 to 78.1. Also, it is evident from a study of Table 1 that there is an appreciable differential in the variability within varieties. In fact it may be seen that the mean values for varietal response are directly related to the magnitude of variability within varietal observations. This relation between varietal means and ranges follows:

	Cook	*Rowden*	*H. & H.*
Mean	2.24	4.75	21.93
Range	26.2	16.0	78.1

Cochran *(3)* has suggested that such a relationship may be taken as a warning that the ordinary combined analysis of variance would be invalid, since it could not be assumed that treatment comparisons all have the same variance.[2] This means that the pooled error variance used in testing mean squares of effects should be segregated into components consistent with homogeniety of effect variances.

In addition, the design of the experiment according to split-plots necessitates the incorporation of suitable modifications in the analysis. In order to illustrate the importance of taking into account peculiarities in the data and in the design, the straightforward analysis is presented, followed with analyses incorporating necessary modifications. Table 3 presents the ordinary, or straightforward, analysis of variance of the data of Table 1, according to the "machine method" described by Snedecor *(7)* and Love *(5)*. The results of this analysis confirm the conclusion that all main effects are significant, which was manifested by visual inspection of the summarized data. In fact, they are all highly significant. The results in Table 3 also indicate that the first order interactions, N × V and P × V, are highly significant, and that the first order interactions, N × K and P × K, reach the 5 per cent level of significance. Instead of going into detail on the interpretation of these findings, it will be more to the point of the discussion to present the analysis according to split-plots for purposes of comparison.

The analysis of variance of Experiment A, according to the split-plot technique, is presented in Table 4. Since the procedure

[2] A X^2 test, given by Clark and Leonard *(2)*, to measure homogeniety of variances substantiated the fact that variety variances were heterogeneous.

TABLE 3. ANALYSIS OF VARIANCE OF DATA IN TABLE 1

Source of variation	Sum of squares	Degrees of freedom	Mean square	F values		
				Found	Required	
					5 per cent	1 per cent
Total	27,403.15	143				
Blocks	256.09	3	85.36	1.35	2.70	3.98
N	1,909.44	2	954.72	15.14**	3.09	4.82
P	2,140.60	1	2,140.60	33.93**	3.94	6.90
N × P	289.48	2	144.74	2.29	3.09	4.82
K	602.70	1	602.70	9.55**	3.94	6.90
N × K	404.45	2	202.22	3.21*	3.09	4.82
P × K	270.60	1	270.60	4.29*	3.94	6.90
N × P × K	92.54	2	46.27			
V	11,025.69	2	5,512.84	87.39**	3.09	4.82
N × V	1,055.48	4	263.87	4.18**	2.46	3.51
P × V	1,755.65	2	877.82	13.92**	3.09	4.82
N × P × V	45.70	4	11.42			
K × V	302.29	2	151.14	2.40	3.09	4.82
N × K × V	333.72	4	83.43	1.32	2.46	3.51
P × K × V	124.71	2	62.35			
N × P × K × V	170.44	4	42.61			
Error	6,623.57	105	63.08[1]			

[1] Difference required for significance between means (shown in Table 2):

Source	*Difference required for odds of:*	
	19:1	*99:1*
Varieties	3.2176	4.2577
Nitrogen levels	3.2176	4.2577
Phosphorus levels	2.6246	3.4731
Potassium levels	2.6246	3.4731

* Low significance.
** High significance.

for this method differs somewhat from the ordinary analysis, a few explanatory comments may be in order. The procedure consists mainly in the calculation of a separate error term for the testing of the various effects confounded with each degree of block subdivision. The first error term [(a) in Table 4] was calculated by assuming that the average of all 12 pots in a nitrogen subblock represents a single observation, making but 12 units in the experiment (nitrogen × blocks) or 11 degrees of freedom. Three degrees of freedom were due to blocks, two to nitrogen levels, and six to block × N level interaction, which is used as the error term to measure significance of blocks and nitrogen levels. Thus, the portion of Table 4 subtended by error term (a) was calculated as follows:

SUMMARIZED DATA

N Level	*Block I*	*Block II*	*Block III*	*Block IV*	*Sum*
Low	102.4	41.2	72.0	56.4	272.0
Medium	112.8	101.5	85.1	122.7	421.1
High	204.3	147.9	200.2	141.9	694.3
Sum	419.5	290.6	357.3	321.0	1,388.4

ANALYSIS OF VARIANCE

Source of variance	*Sum of squares*	*Degrees of freedom*	*Mean square*
Total	2,421.13	11	
Blocks	256.09	3	85.36
N level	1,909.44	2	954.72**
Error (a)	255.60	6	42.60

The second error term [(b) in Table 4] was derived by taking the six pots in each phosphorus subblock within the nitrogen subblocks as a unit, making 24 units in the experiment or a total of

TABLE 4. ANALYSIS OF EXPERIMENT A ACCORDING TO SPLIT-PLOT TECHNIQUE

Source of variation	Sum of squares	Degrees of freedom	Mean square	F values		
				Found	Required	
					5 per cent	1 per cent
Total N × B	2,421.13	11				
Blocks	256.09	3	85.36	2.00	4.76	9.78
N	1,909.44	2	934.72	22.41**	5.14	10.92
Error (a)	255.60	6	42.60			
Total N × P × B	5,474.06	23				
Less total N × B	3,052.93	12				
P	2,140.60	1	2,140.60	30.93**	5.12	10.56
N × P	289.48	2	144.74	2.09	4.26	8.02
Error (b)	622.85	9	69.21			
Total N × P × K × B	7,935.97	47				
Less total N × P × B	2,461.91	24				
K	602.70	1	602.70			
N × K	404.45	2	202.22	9.94**	4.41	8.28
P × K	270.60	1	270.60	3.33	3.55	6.01
N × P × K	92.54	2	46.27	4.46**	4.41	8.28
Error (c)	1,091.62	18	60.65			
Total N × P × K × V × B	27,403.15	143				
Less total N × P × K × B	19,467.18	96				
V	11,025.69	2	5,512.84	85.30**	3.13	4.92
N × V	1,055.48	4	263.87	4.08**	2.50	3.60
P × V	1,755.65	2	877.82	13.58**	3.13	4.92
N × P × V	45.70	4	11.42			
K × V	302.29	2	151.14	2.34	3.13	4.92
N × K × V	333.72	4	83.43	1.29	2.50	3.60
P × K × V	124.71	2	62.35			
N × P × K × V	170.44	4	42.61			
Error (d)	4,653.50	72	64.63			

** High significance.

23 degrees of freedom. Eleven of these latter 23 degrees of freedom have already been accounted for in testing nitrogen levels. Thus, after deducting the value for the sums of squares (2,421.13), associated with the 11 degrees of freedom used in testing nitrogen levels, from the value for the total sums of squares (5,474.06), associated with the 24 phosphorus subblocks, there remains the value (3,052.93), which is the sum of squares for the 12 degrees of freedom used in testing P levels and N $\times$ P interaction. Potassium effects were tested by using the total sums of squares (7,935.97) associated with the 47 degrees of freedom of the 48 potassium subblocks, after deducting the sums of squares (5,474.06) associated with the 23 degrees of freedom already accounted for by N, P, and block effects. This leaves a value of 2,461.91 as the sums of squares associated with the 24 degrees of freedom for testing potassium effects. Variety effects are similarly tested. Since the individual pots now become the unit subblocks, there will be $143 - 47 = 96$ degrees of freedom for testing variety effects.

It is now apparent that the error terms used in testing the effects associated with each degree of subdivision in the experiment may vary considerably. Their magnitude in each case is dependent upon the accuracy with which the effects associated with each degree of subdivision are estimated. For example, the error term for testing N has a value of 42.60, whereas the one for testing P has a value of 69.21. This means that the replicate nitrogen subblocks were more uniform in observed response than the replicate phosphorus subblocks. When the pooled error term of Table 3, with a value of 63.08, is used for testing the mean squares of effects, this relative accuracy is not taken into account. Hence, it is conceivable that the variability in observed effects of one subdivision may have been sufficient to obliterate the actual uniformity within another subdivision had the pooled error term been used. Split-plot technique obviates the possibility that the uniformity of observations in one degree of subdivision will be jeopardized in the test for significance by extraneous variability in another degree of subdivision. However, this particular advantage of the split-plot technique may be, and in most instances is, more than counteracted by the loss in precision for measuring the significance of effects confounded with the first degree of subdivision. Thus, in the data there are only six degrees of freedom associated with the error term for measuring nitrogen effects by the split-plot technique, whereas there are 105 degrees of

freedom associated with the pooled error term of Table 2. Consequently, the latter procedure requires an F value of only 4.82 for significant odds of 99:1, as contrasted with a required F value of 10.92 for the same odds by the split-plot procedure. Under many experimental conditions this might result in the loss of valuable information on some effects. If the experimenter finds it desirable to use this type of experimental design, it would be advisable, if possible, to confound those effects that are expected to give the most marked divergence in response with the primary subdivisions of the main blocks or those comparisons in which the investigator feels the loss in precision can be sacrificed. It might be emphasized at this point that the present tendency, with plant nutrition work in the greenhouse, to carry out experiments in large tanks of nutrient solution, or benches of sand supplied with a uniform nutrient solution, may require the use of split-plot analysis such as exemplified herein. Regardless of the number of plants in a tank or sand bench, the entire tank or bench has to be taken as the statistical unit as far as nutrition treatments are concerned. However, each tank or bench, which may be too large or too small for complete blocks, may contain several varieties, types of pruning, degrees of light intensity, or other variable factors. Under such experimental conditions, the split-plot type of design and analysis may prove quite valuable if it is properly designed.

It is admitted that no real difference in the results of this experiment was occasioned by the two methods of analysis. The only difference is the failure of the N × K interaction to attain significance by split-plot analysis, whereas it attained the 5 per cent level of significance when the pooled error term was used. However, in order to take into account variety variances mentioned earlier, it is necessary to introduce a modification into the conventional split-plot analysis of Table 4. The two degrees of freedom for variety comparisons may be segregated into individual components: one for the comparison between Cook and Rowden varieties, which are homogeneous, and the other for the comparison between Half & Half and the mean of the other two. Since this modification involves only that section of Table 4 dealing with variety comparisons, as measured with error term (d), Table 5 is confined to the representation of this section with variety comparisons segregated into V_1 (Cook *vs.* Rowden) and V_2 (Half & Half *vs.* Cook + Rowden), as tested by error terms

(d_1) and (d_2). In the calculation of mean squares involving V_1, only the data for Cook and Rowden in Table 1 are used, with commensurate allowance being made for associated nutritional effects and their errors, as in the combined analysis of Table 4. These data for Cook and Rowden are analyzed according to the following summary:

Source of variation	*Sum of squares*	*D. F.*	*Notes*
Total	2,311.45	95	
N × P × K × B	1,303.22	47	Allowance already made for this source of variation in combined analysis
Difference	1,008.21	48	
V_1 effects	388.71	12	Sum of V_1 and all interactions involving V_1, which are segregated according to usual procedure
Error (d_1)	619.50	36	

It should be emphasized that allowance must be made for nutrient effects and their associated errors within the Cook and Rowden data before segregation of V_1 effects, since this source of variation has been taken into account in the combined analysis in Table 4.

Mean squares involving V_2 were derived by entering each observation for Half & Half variety into the calculations twice to counterbalance the two associated observations for Cook and Rowden varieties. This modification in handling the data requires that commensurate divisors and correction terms be used in the calculations.[3] Allowance must also be made for associated

[3] Formulation:

In the following C stands for Cook, R for Rowden, H for Half & Half observations, and (C + R) the sum of paired Cook and Rowden observations.

$$\text{Total sums of squares} = \frac{\Sigma (C + R)^2 + \Sigma (2H)^2}{(1^2 + 2^2 + 1^2)\frac{1}{2}} - \frac{[\Sigma (C + R + 2H)]^2}{(1^2 + 2^2 + 1^2)48}$$

$$\text{Total } N \times P \times K \times B = \frac{\Sigma (C + R + 2H)^2}{1^2 + 2^2 + 1^2} - \frac{[\Sigma (C + R + 2H)]^2}{(1^2 + 2^2 + 1^2)48}$$

$$V_2 = \frac{[\Sigma (C + R)]^2 + [\Sigma (2H)]^2}{(1^2 + 2^2 + 1^2)48/2} - \frac{[\Sigma (C + R + 2H)]^2}{(1^2 + 2^2 + 1^2)48}$$

In the denominators of all V_2 formulations, the expression, $(1^2 + 2^2 + 1^2)$, is substituted for the figure 2, or $(1^2 + 1^2)$, used in V_1 formulations. This modification is further exemplified under Experiment B. The figure, 48, represents the number of observations within each variety.

nutritional effects and their errors as the following summary indicates:

Source of variation	*Sum of squares*	*D. F.*	*Notes*
Total	30,700.22	95	
N × P × K × B	12,241.00	47	Allowance already made for this source of variation
Difference	18,459.22	48	
V_2 effects	14,424.94	12	Sum of V_2 and all interactions involving V_2, which are segregated according to usual procedure
Error (d_2)	4,034.28	36	

The wide differential between error terms (d_1) and (d_2) is the striking feature of Table 5. The error variance for testing V_2 effects is over six times as large as the error variance for testing V_1 effects. Although both V_1 and V_2 effects are highly significant, it is evident that V_1 would not have attained significance if it had been measured by the combined error term for variety

TABLE 5. SEGREGATION OF VARIETY COMPARISONS OF TABLE 4 INTO COMPONENTS V_1 AND V_2

Source of variation	Sum of squares	Degrees of freedom	Mean square	F values		
				Found	Required	
					5 per cent	1 per cent
Total N × P × K × V × B	27,403.15	143				
Less total N × P × K × B	19,467.18	96				
V_1	151.25	1	151.25	8.79**	4.12	7.42
N × V_1	27.09	2	13.54		3.27	5.28
P × V_1	23.50	1	23.50	1.37		
V × P × V_1	22.82	2	11.41			
K × V_1	10.07	1	10.07			
N × K × V_1	81.52	2	40.76	2.37		
P × K × V_1	1.06	1	1.06			
N × P × K × V_1	71.40	2	35.70	2.07		
Error (d_1)	619.50	36	17.21			
V_2	10,874.44	1	10,874.44	97.04**	4.12	7.42
N × V_2	1,028.39	2	514.19	4.59*	3.27	5.28
P × V_2	1,732.15	1	1,732.15	15.46**		
N × P × V_2	22.88	2	11.44			
K × V_2	292.22	1	292.22	2.61		
N × K × V_2	252.20	2	136.10	1.21		
P × K × V_2	123.64	1	123.64	1.10		
N × P × K × V_2	99.02	2	45.51			
Error (d_2)	4,034.28	36	112.06			

* Low significance.
** High significance.

variances (Table 4), instead of an error term that was not inclusive of the marked variability within the V_2 comparisons. The analytical results in both Tables 3 and 4 indicated that interactions V × P and V × N were highly significant, showing that wilt development within the various varieties was not uniform for various levels of either phosphorus or nitrogen supply. Table 5 brings out the fact that neither P × V_1 nor N × V_1 is significant, and N × V_2 attains low significance. This indicates that there was no interdependence between variety and level of nutrients upon wilt development within the two more resistant varieties. The interdependence between these factors, which was shown by Table 4, was entirely due to the disparity of wilt development as influenced by levels of nutrients between Half & Half variety and the mean of the two more resistant varieties. However, the analyses show that potassium acted independent of varietal influence, regardless of segregation of varietal contributors.

In summarizing the analysis of Experiment A, it is evident that all main contributors are highly significant, a deduction that was apparent in Table 2. Interactions P × K and N × V_2 reached the 5 per cent level and interaction P × V_2 reached the one per cent level of significance, indicating that these respective pairs of factors were not acting independently upon disease development. Any semblance of disparity induced by other combinations of factors may be regarded as fortuitous. Undoubtedly, the finding of most of these interactive effects to be without significance is a definite contribution to the interpretation of the experiment, inasmuch as a study of the raw data might have suggested otherwise.

This discussion of interactive effects has all been based upon parity of arithmetical differences as the basis of independence (lack of interaction). Cochran *(3)* has pointed out that the definition of independence of related factors, which is expressed by the usual test for interactions, may not be the one that will give the desired interpretation of results. In this particular experiment it was felt that independence should be considered as indicative of disproportionate responses, instead of indicating actual algebraic differences in response, as is the case in the usual test for interaction. For example: Increase in the supply of a certain nutrient might cause increase in disease in a resistant variety from 4 per cent to 8 per cent, and an increase in a susceptible variety from 40 per cent to 80 per cent. The straightforward analysis of variance would undoubtedly show these differences

in increase (4 per cent and 40 per cent) to be a significant interaction; whereas, in relation to disease potentialities of the two varieties, the differences are merely proportionate since each shows the same percentage of increase (100 per cent).

The original data may be easily transformed so that independence of any treatment effect can be measured against base levels for varieties. This was done for the original data of Experiment A by dividing the value for each observation within a variety by the total of all observations for that variety. Each new value then becomes a percentage of the total observed disease within each variety, and the sums for each variety become 100. Table 6 presents the summaries for the various main effects and first order interactions after proportionate treatment with varieties as base levels. Thus, with the marked disparity in varietal response removed, it can be seen by inspection whether or not there is actually any difference in the proportion of nutrient response in different varieties (nutrient · variety interactions), since, following this treatment, differences in proportionality will be revealed as actual differences in summation, or in mean responses. In Table 6 it is obvious that there is no difference in

TABLE 6. SUMMARY TABLES FOR ALL FIRST ORDER INTERACTIONS OF DATA AFTER EACH OBSERVATION IS MADE PROPORTIONATE TO RESPECTIVE VARIETAL MEANS

Nutrient level	ΣC'ook	ΣRowden	ΣHalf & Half	Mean of V
ΣN_1	17.20	18.53	20.03	18.58
ΣN_2	22.01	24.89	32.44	26.44
ΣN_3	60.77	56.54	47.49	54.93
ΣP_1	29.73	30.02	30.00	29.91
ΣP_2	70.25	69.94	69.96	70.05
ΣK_1	80.39	57.50	59.24	65.71
ΣK_2	19.59	42.46	40.72	34.25

Nutrient level	ΣN_1	ΣN_2	ΣN_3	Mean of N
ΣP_1	22.67	24.21	42.87	29.91
ΣP_2	33.09	55.13	121.93	70.05
ΣK_1	31.18	48.48	117.47	65.71
ΣK_2	24.58	30.86	47.33	34.25

Nutrient level	ΣP_1	ΣP_2	Mean of P
ΣK_1	53.05	144.08	98.56
ΣK_2	36.70	66.07	51.38
Mean of K	44.87	105.07	

TABLE 7. ANALYSIS OF EXPERIMENT A ACCORDING TO SPLIT-PLOT TECHNIQUE AFTER PROPORTIONATE TREATMENT WITHIN VARIETIES

Source of variation	Sum of squares	Degrees of freedom	Mean square	F values		
				Found	Required	
					5 per cent	1 per cent
Total N × B	180.077	11				
Blocks	26.337	3	8.779	3.17	4.76	9.78
N	137.147	2	68.573	24.80**	5.14	10.92
Error (a)	16.593	6	2.765			
Total N × P × B	366.961	23				
Total less N × B	186.884	12				
P	100.668	1	100.668	26.27**	5.12	10.56
N × P	51.731	2	25.865	6.75*	4.26	8.02
Error (b)	34.485	9	3.832			
Total N × P × K × B	601.265	47				
Less total N × P × B	234.304	24				
K	61.832	1	61.832	12.03**	4.41	8.28
N × K	48.035	2	24.017	4.67*	3.55	6.01
P × K	26.402	1	26.407	5.14*	4.41	8.28
N × P × K	15.493	2	7.746	1.51	3.55	6.01
Error (c)	92.542	18	5.141			
Total N × P × K × V × B	1,183.457	143				
Less total N × P × K × B	582.192	96				
V_1	.000	1				
N × V_1	.874	2	.437			
P × V_1	.004	1	.004			
N × P × V_1	2.966	2	1.483			
K × V_1	21.812	1	21.812	2.55	4.12	7.42
N × K × V_1	59.147	2	29.573	3.46*	3.27	5.28
P × K × V_1	5.891	1	5.891			
N × P × K × V_1	37.754	2	18.877	2.21	3.27	5.28
Error (d$_1$)	307.418	36	8.539			
V_2	.000	1				
N × V_2	8.757	2	4.378	1.69	3.27	5.28
P × V_2	.001	1	.001			
N × P × V_2	16.105	2	8.052	2.73	3.27	5.28
K × V_2	5.227	1	5.297	1.77	4.12	7.42
N × K × V_2	4.956	2	2.478			
P × K × V_2	2.181	1	2.181			
N × P × K × V_2	2.761	2	1.380			
Error (d$_2$)	106.338	36	2.954			

* Low significance.
** High significance.

proportionate response to increase in level of P supply in the three varieties, since the actual differences shown (after proportionate treatment) are nearly identical: 40.52, 39.92, and 39.96 for Cook, Rowden, and Half & Half, respectively. It would have been difficult to arrive at this conclusion by the mean responses in

the original data, Table 2. In Table 2 the differences due to increase in level of P supply are quite different and it would be difficult to decide if they were or were not disproportionate, actual differences being 1.80, 3.80, and 17.52 for Cook, Rowden, and Half & Half, respectively, with V × P interaction being highly significant.

The split plot analysis of the data, expressed as proportions, is shown in Table 7. Since the sums for each variety are 100 per cent, the mean square for variety differences must be zero. However, the differences due to nutritional treatment within each variety are all measured within the same normal population, and the pooled effect of nutrients becomes a more uniformly weighted effect of the three varieties, and does not show, as does the analysis of the original data (Tables 4 and 5), the pooled effect of nutrients to be mostly that within the susceptible variety — that within the resistant varieties being obscured because of the relative magnitude of the differences. Furthermore, the interpretation for interaction is based on a different definition, particularly in relation to varieties. It may now be stated that treatments causing the same proportionate response in different varieties (nutrient × variety interactions) are acting independently of varietal influence.

The interpretation of interactions N × P, N × K, and P × K (interactions not involving V), however, follow rather closely those that would be developed on the untransformed data with the exception that, in the pooling of variety observations, greater weight is placed on those for the variety showing least differences on the original scale, and *vice versa*. That is, proportionate treatment within varieties does not alter the relative magnitude of nutrient differences giving rise to nutrient interactions, but it does avoid the contingency of large differences in effects within the susceptible variety, almost completely obscuring the small differences within the resistant variety.

The results of an analysis are definitely influenced by proportional treatment of the data. To be sure, with the exception of variety comparisons, all main contributors continue to show a highly significant effect upon the results. All first order interactions between nutrient effects were found to be significant, whereas only interaction P × K attained significance on analysis of the unmodified data. Evidently, the readjustment of treatment differences to a comparable basis within each variety brings out the interrelationship of nutrients which was masked by the

wide differentials in nutrient effects between varieties. Thus, the relative amount of wilt development in all varieties, as influenced by a given nutrient element, was modified by the supply level of the other two variable nutrients. Only one interaction involving varietal comparisons attained significance, $N \cdot K \cdot V_1$. This indicates that, with one exception, varietal responses to nutrients were entirely within the realm of statistically commensurate proportions.

Each experiment must be analyzed according to its design and inherent characteristics of the data. In Experiment A, the split-plot design and the heterogeneity of errors of observation among varieties necessitated an analysis according to split-plots with the segregation of varietal comparisons. Transforming the data to a proportionate basis prior to the analysis facilitated interpretations by placing treatment responses upon a comparable basis between varieties, and by placing varietal responses to treatments upon a proportional relationship. However, since proportionate treatment of data has not as yet received wide application, (its value and limitations are not known), for the present it is preferable that it be used as an auxiliary method to interpret relationships.

EXPERIMENT B

The discussion of Experiment A considered that, although the design of plant nutrition experiments in the greenhouse may often necessitate the use of split-plots and partial disruption of blocks, this does not militate against the use of analysis of variance if conducted with proper evaluation of those effects that are thus confounded with block subdivisions. The design of Experiment B was also unorthodox as to arrangement of component blocks, and as to confounding of certain effects with block subdivisions, thus presenting another example of how the split-plot type of experiment may be properly analyzed. In addition, this experiment is utilized to demonstrate the possibilities of interpretation in a factorial experiment in which time effects are analyzed and the proper method for making such analyses is discussed. The use of analysis by single degrees of freedom is introduced here because of the opportunity to segregate multiple degree-of-freedom factors (more than two levels) into linear and curvilinear comparisons. Restratification of the partitioned error variances is discussed briefly.

This experiment involved a study of the rate of development of fusarium wilt in three varieties of cotton plants grown in

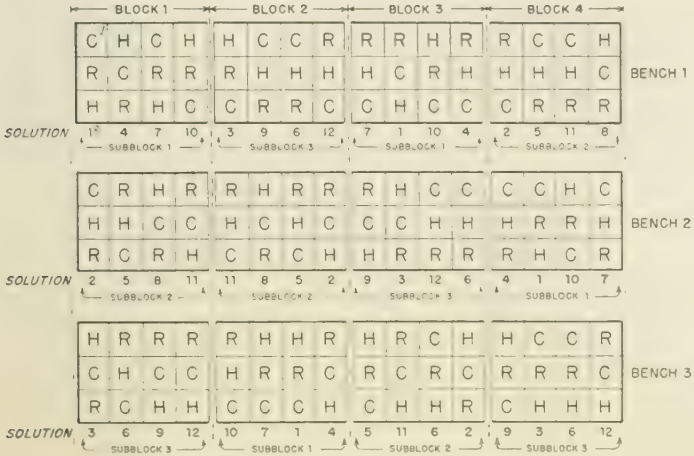
BLOCK 1
BLOCK 2
BLOCK 3
BLOCK 4
C H C H H C C R R R H R R C C H
R C R R R H H H H C R H H H H C
H R H C C R R C C H C C C R R R
BENCH 1
SOLUTION 1 4 7 10 3 9 6 12 7 1 10 4 2 5 11 8
SUBBLOCK 1 SUBBLOCK 3 SUBBLOCK 1 SUBBLOCK 2
C R H R R H R R R H C C C C H C
H H C C H C H C C C H H H R R H
R C R H C R C H H R R R R H C R
BENCH 2
SOLUTION 2 5 8 11 11 8 5 2 9 3 12 6 4 1 10 7
SUBBLOCK 2 SUBBLOCK 2 SUBBLOCK 3 SUBBLOCK 1
H R R R R H H R H R C H H C C R
C H C C H R R C R C R C R R R C
R C H H C C C H C H H R C H H H
BENCH 3
SOLUTION 3 6 9 12 10 7 1 4 5 11 6 2 9 3 6 12
SUBBLOCK 3 SUBBLOCK 1 SUBBLOCK 2 SUBBLOCK 3

Figure 2. ARRANGEMENT OF CULTURES IN EXPERIMENT B.

[1] Key to varieties (each letter designates one jar):
C = Cook 307
R = Rowden 2088
H = Half & Half

[2] Key to treatments:

1 = Low NO_3, pH 4	5 = High NO_3, pH 6	9 = Low NH_4, pH 8
2 = Low NO_3, pH 6	6 = High NO_3, pH 8	10 = High NH_4, pH 4
3 = Low NO_3, pH 8	7 = Low NH_4, pH 4	11 = High NH_4, pH 6
4 = High NO_3, pH 4	8 = Low NH_4, pH 6	12 = High NH_4, pH 8

sand culture in the greenhouse. The plants of each variety were supplied with 12 different nutrient solutions, varied as to source of nitrogen (nitrate *vs.* ammonium), level of nitrogen supply (20 *vs.* 100 parts per million), and pH (4.0, 6.0, and 8.0). The code values of disease intensity were taken at 31, 40, and 49 days after planting. Thus, the experiment is characterized by a $3 \times 3 \times 2 \times 2$ design, involving three Varieties, three Acidity intensities, two Sources of nitrogen, two Levels of nitrogen supply, with the additional complication of three Times of observation.[1] There were four replicates in the experiment, each replicate comprising a complete block.

The actual arrangement of the individual pots of plants on three benches in the greenhouse is shown in Figure 2. A consideration of this design entirely from the statistical point of view will reveal a few undesirable features. Each of the four blocks

[1] Hereinafter, the single capital letter will be used to designate each factor.

were extended laterally across the three benches. It might have been preferable to have had each block on a single bench, but the available space and the arrangement of this space in a greenhouse is seldom optimal for every experimental purpose. In this particular case a gradient along the benches in light or temperature would be better evaluated by the plan used (blocks across the three benches) than by the alternate plan (each block extending along one bench). Had the gradient been across the benches, then the converse design would have been best for taking out block or replicate variability. Advance information on the direction of probable gradient effects was incomplete, and the plan that facilitated subdivision into four replicate blocks was chosen. Subblocks were confounded with a main effect, an admittedly undesirable feature. In this experiment, as in the preceding one, each tier of three pots across the bench received identical nutrient treatment in order to obviate errors in nutrient application. Even though the three varieties of cotton plants in the three pots of each tier were randomized for position within the tier, the restriction of nutrient treatments was contrary to the precepts of ideal arrangement, necessitating the analysis of nutrient effects by the split-plot technique. It is the experience of the writers in their plant nutrition work that it is far more essential to avoid errors of manipulation than to eliminate a rather dubious deficiency in the attainment of a theoretically ideal design.

Analysis of Experiment B

The time factor. The observations for disease intensity made 31 days after planting are presented in Table 8a, those at 40 days in Table 8b, and those at 49 days in Table 8c. There are

Table 8A. Disease Values of Plants at 31 Days After Planting

Solution No.	Block I			Block II			Block III			Block IV		
	C[1]	R[1]	H[1]	C	R	H	C	R	H	C	R	H
1	0.0	0.0	8.7	0.0	0.0	0.0	0.0	0.0	3.7	0.0	0.0	0.0
2	.0	.0	.0	.0	.0	.0	.0	.0	3.7	.0	.0	.0
3	.0	.0	8.7	.0	.0	12.5	.0	.0	.0	.0	.0	6.6
4	.0	.0	7.5	.0	.0	.0	.0	.0	11.2	.0	.0	12.5
5	.0	.0	12.5	.0	.0	16.5	.0	.0	7.5	.0	.0	.0
6	.0	.0	8.7	.0	3.7	.0	.0	.0	.0	.0	.0	3.7
7	.0	.0	3.7	.0	.0	12.5	.0	.0	.0	.0	.0	.0
8	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	7.5
9	.0	.0	7.5	.0	3.7	.0	.0	.0	30.0	.0	.0	3.7
10	.0	.0	16.2	.0	.0	7.5	7.5	.0	21.2	.0	.0	.0
11	.0	.0	12.5	.0	.0	12.5	.0	.0	.0	.0	3.7	12.8
12	.0	.0	20.0	.0	.0	46.2	3.7	12.5	28.7	.0	.0	23.7

[1] C = Cook 307; R = Rowden 2088; H = Half & Half.

TABLE 8B. DISEASE VALUES OF PLANTS AT 40 DAYS AFTER PLANTING

Solution No.	Block I			Block II			Block III			Block IV		
	C[1]	R[1]	H[1]	C	R	H	C	R	H	C	R	H
1	3.0	3.7	20.0	0.0	0.0	7.5	0.0	0.0	8.7	0.0	0.0	7.5
2	.0	.0	16.2	.0	.0	12.5	.0	.0	28.7	.0	.0	3.7
3	.0	.0	12.5	.0	.0	37.5	.0	3.7	8.7	.0	8.7	17.7
4	.0	.0	55.0	.0	.0	38.7	.0	.0	37.5	3.7	4.2	46.2
5	.0	8.7	57.5	.0	.0	28.7	.0	3.7	50.0	.0	.0	12.5
6	3.7	16.2	37.5	3.7	17.5	30.0	.0	.0	.0	.0	12.5	7.5
7	.0	.0	12.5	.0	.0	21.2	.0	.0	8.7	.0	.0	3.7
8	.0	12.5	28.7	.0	.0	66.2	.0	.0	50.0	.0	.0	28.7
9	.0	.0	41.2	.0	3.7	8.7	.0	.0	37.5	.0	3.7	33.7
10	.0	.0	78.7	.0	.0	75.0	20.0	3.7	87.5	3.7	.0	85.0
11	.0	16.2	87.5	20.0	3.7	75.0	.0	.0	60.0	4.2	25.0	85.7
12	4.2	12.5	96.2	3.7	7.5	87.5	3.7	30.0	75.0	3.7	7.5	75.0

[1] C = Cook 307 ; R = Rowden 2088 ; H = Half & Half.

certain striking features in these tables that could not be made more obvious by any statistical method. It is evident from Table 8a that, at 31 days after planting, only a very few of the Cook or Rowden plants showed any evidence of disease symptoms, whereas 65 per cent of the cultures containing Half & Half plants showed some degree of wilt development at this time. The marked differential in rate of development of disease symptoms between Half & Half and the other two varieties is obvious. (All plants were inoculated with a suspension of wilt mycelium and spores at the same time.) Even at 40 days after planting (Table 8b), only one-third of the 96 cultures of Cook and Rowden varieties together showed any symptoms of disease, whereas only one of the 48 cultures of Half & Half showed no wilt symptoms, and in several cultures most of the plants were dead. (A code value of 100 indicates all plants dead.) In other words, during the 9-day interval between the observations at 31 and 40 days, disease intensity ob-

TABLE 8C. DISEASE VALUES OF PLANTS AT 49 DAYS AFTER PLANTING

Solution No.	Block I			Block II			Block III			Block IV		
	C[1]	R[1]	H[1]	C	R	H	C	R	H	C	R	H
1	12.5	3.7	71.2	3.7	7.5	45.0	0.0	0.0	25.0	3.7	28.7	52.5
2	.0	.0	33.7	.0	.0	37.5	.0	8.7	50.0	.0	.0	53.7
3	.0	7.5	42.5	.0	.0	53.7	.0	7.5	28.7	3.7	16.2	36.6
4	.0	16.2	70.0	3.7	7.5	71.2	3.7	7.5	78.7	11.2	4.2	96.2
5	3.7	25.0	75.0	.0	7.5	70.0	.0	8.7	71.2	.0	7.5	71.2
6	38.7	25.0	58.7	3.7	30.0	45.0	25.0	7.5	20.0	3.7	16.2	18.7
7	.0	21.2	23.7	.0	.0	37.5	.0	3.7	37.5	.0	3.7	16.2
8	.0	12.5	57.5	.0	7.5	78.7	.0	.0	85.0	.0	.0	50.0
9	.0	3.7	45.0	.0	3.7	33.7	.0	3.7	58.7	.0	7.5	41.2
10	37.5	.0	100.0	7.5	12.2	100.0	50.0	25.0	100.0	25.0	3.7	100.0
11	37.5	32.5	100.0	45.0	37.5	96.2	50.0	.0	96.2	14.2	50.0	100.0
12	8.5	37.5	100.0	32.5	11.2	100.0	7.5	41.2	100.0	8.7	20.0	100.0

[1] C = Cook 307 ; R = Rowden 2088 ; H = Half & Half.

viously developed much more rapidly in Half & Half plants than in plants of the other two varieties. A comparison between Tables 8b and 8c will reveal that, during the 9-day interval between observations at 40 and 49 days, there had been an appreciable increase in wilt intensity in the Cook and Rowden varieties, 65 per cent of the cultures showing symptoms at the later date. There was an increase in disease intensity in Half & Half plants during this same interval, but cultures in which nearly all plants were dead at the 40-day count had little opportunity to show an increase in disease intensity at the next count.

Although the foregoing only touches upon the information embodied in Tables 8a, 8b, and 8c, it does emphasize the importance of taking into account the time factor in experiments of this nature. The relationship between time of observation and treatment effects is not as obvious between time and variety. A more complete comprehension of the information present in the data may be gained from Tables 9a, 9b, and 9c, in which are presented the mean values for all main effects and first order interactions of the data presented in Tables 8a, 8b, and 8c, respectively.

A thorough inspection of these values reveals certain factors to be effective beyond all reasonable doubt. Other relationships invoke varying degrees of uncertainty as to their relative significance in interpreting the results of this experiment. It is the

TABLE 9A. MEANS OF DISEASE CODE VALUES FOR ALL MAIN EFFECTS AND FIRST ORDER INTERACTIONS FOR DATA TAKEN AT 31 DAYS (TABLE 8A)

Variety	pH 4	pH 6	pH 8	NO_3-N	NH_4-N	Low N	High N	Mean
Cook 307	0.47	0.00	0.25	0.00	0.47	0.00	0.47	0.23
Rowden 2088	.00	.28	1.24	.15	.83	.15	.83	.48
Half & Half	6.54	5.57	12.50	5.17	11.25	4.55	11.88	8.20
Mean	2.34	1.93	4.66	1.77	4.18	1.56	4.39	2.97

Acidity	NO_3-N	NH_4-N	Low N	High N
pH 4	1.82	2.86	1.19	3.48
pH 6	1.68	2.20	.47	3.40
pH 8	1.83	7.49	3.03	6.29
Mean	1.78	4.18	1.56	4.39

N source	Low N	High N
NO_3-N	1.22	2.33
NH_4-N	1.91	6.46
Mean	1.56	4.39

TABLE 9B. MEANS OF DISEASE CODE VALUES FOR ALL MAIN EFFECTS AND FIRST ORDER INTERACTIONS FOR DATA TAKEN AT 40 DAYS (TABLE 8B)

Variety	pH 4	pH 6	pH 8	NO_3—N	NH_4—N	Low N	High N	Mean
Cook 307	1.94	1.51	1.42	0.62	2.63	0.15	3.10	1.62
Rowden 2088	.73	4.36	7.72	3.29	5.25	1.50	7.04	4.27
Half & Half	37.09	43.23	37.89	24.26	54.54	21.75	57.05	39.40
Mean	13.25	16.37	15.68	9.39	20.81	7.80	22.40	15.10

Acidity	NO_3—N	NH_4—N	Low N	High N
pH 4	9.85	16.65	4.05	22.45
pH 6	9.26	23.47	10.30	22.43
pH 8	9.06	22.29	9.05	22.30
Mean	9.39	20.80	7.80	22.39

N source	Low N	High N
NO_3—N	5.58	10.02
NH_4—N	13.19	31.59
Mean	9.39	20.81

presence of numerous relationships of the latter class that justify the application of statistical methods to the data. For example, it is evident in Table 9a that the disparity in wilt development at the first count (31 days) between Half & Half plants and those of the other two varieties is beyond the necessity for statistical proof. Also, it is evident that differences between the means for sources of nitrogen and between the means for levels of nitrogen are significant contributors to variability. It is apparent that acidity levels and the interaction, (sources of N) × (levels of N), may also be significant contributors. Even though other interrelationships between factors appear evident, it is not possible to arrive at any definite conclusion without statistical treatment. Similar deductions may be made by inspecting the means in Tables 9b and 9c, but here, also, certain observed effects may be open to question unless significance is established by means of statistical analysis.

A comparison of the mean values for the three separate recordings in Tables 9a, 9b, and 9c will disclose certain obvious relationships, but it will be found much easier to make such deductions if these means are retabulated. It was pointed out in connection with the raw data in Tables 8a, 8b, and 8c that the time factor was involved in the appearance of wilt symptoms among the various varieties. This fact is made more obvious by com-

TABLE 9C. MEANS OF DISEASE CODE VALUES FOR ALL MAIN EFFECTS AND FIRST ORDER INTERACTIONS FOR DATA TAKEN AT 49 DAYS (TABLE 8C)

Variety	pH 4	pH 6	pH 8	NO_3—N	NH_4—N	Low N	High N	Mean
Cook 307	9.91	9.40	8.25	4.87	13.50	0.98	17.39	9.19
Rowden 2088	9.05	12.34	14.90	10.11	14.08	6.13	18.07	12.10
Half & Half	64.04	70.37	55.16	53.17	73.21	45.62	80.76	63.19
Mean	27.67	30.70	26.10	22.72	33.60	17.58	38.74	28.16

Acidity	NO_3—N	NH_4—N	Low N	High N
pH 4	25.98	29.35	16.54	38.79
pH 6	21.81	39.59	19.78	41.62
pH 8	20.36	31.85	16.40	35.80
Mean	22.72	33.60	17.57	38.74

N source	Low N	High N
NO_3—N	17.60	27.84
NH_4—N	17.55	49.64
Mean	17.57	38.74

paring the magnitudes of given means at the respective times of observation. This may be better shown as follows:

	Observations at:				
Variety	*31 days*		*40 days*		*49 days*
Cook 307	0.23	(+ 1.39)	1.62	(+ 7.57)	9.19
Rowden 2088	.49	(+ 3.78)	4.27	(+ 7.83)	12.10
Half & Half	8.20	(+ 31.20)	39.40	(+ 23.79)	63.19
Mean	2.97	(+ 12.13)	15.10	(+ 13.06)	28.16

The definite influence of time of observation upon the mean wilt value is obvious, and it is also evident that development was nearly linear with time, a mean increase in wilt value of 12.13 occurring during the first 9-day interval, and one of 13.06 during the second interval. Yet, wilt development within the respective varieties is not linear with time. The mean increment within the Half & Half variety for the first interval was appreciably greater than that during the second interval; and, conversely, the mean increments of wilt development within Cook and Rowden varieties were much smaller during the first interval than they were during the second interval. This reemphasizes the interrelationship of time of observation and varietal specificity as to their influence on wilt development. Although the importance of the time factor with reference to the influence of nutritional factors could be indicated from a study of summary tables, a comparison

of the results of analyses of variance of these data, given respectively in Tables 10a, 10b, and 10c, will present a more explicit picture.

In carrying out analyses on the data of Experiment B it is necessary to recall that acidity levels were confounded with subblocks that were randomized within the main blocks, and that the source × level of nitrogen combinations were confounded with split-plots within the "acidity" subblocks. Each of the three varieties were assigned at random as to position within a given tier

TABLE 10A. ANALYSIS OF VARIANCE OF DATA TAKEN AT 31 DAYS (TABLE 8A)

Source of variation	Sum of squares	Degrees of freedom	Mean square	F values		
				Found	Required	
					5 per cent	1 per cent
Total A × B	343.25	11	0.03			
Blocks	51.09	3	17.03	1.21	4.76	9.78
A	207.39	2	103.69	7.34*	5.14	10.92
Error (a)	94.77	6	14.13			
Total A × S × L × B	1,846.09	47				
Less total A × B	1,502.84	36				
S	208.56	1	208.56	9.60**	4.21	7.88
A × S	191.92	2	95.96	4.42*	3.35	5.49
L	288.15	1	288.15	13.27**		
A × L	5.82	2	2.91			
S × L	106.60	1	106.60	4.91*		
A × S × L	115.26	2	57.63	2.65		
Error (b)	596.53	27	221.72			
Total A × S × L × V × B	6,655.73	143				
Less total A × S × L × B	4,809.64	96				
V_1	1.60	1	1.60		4.11	7.39
A × V_1	8.79	2	4.39	2.41	3.26	5.25
S × V_1	.26	1	.26			
A × S × V_1	4.34	2	2.17	1.19		
L × V_1	.26	1	.26			
A × L × V_1	4.34	2	2.17	1.19		
S × L × V_1	.07	1	.07			
A × S × L × V_1	2.19	2	1.09			
Error (c_1)	65.53	36	1.82			
V_2	1,968.78	1	1,968.78	55.71**	4.11	7.39
A × V_2	249.56	2	124.78	3.53*	3.26	5.25
S × V_2	242.74	1	121.38	3.43		
A × S × V_2	285.16	2	142.58	4.03		
L × V_2	367.21	1	367.21	10.39**		
A × L × V_2	9.37	2	4.68			
S × L × V_2	296.34	1	296.34	8.39**		
A × S × L × V_2	30.85	2	15.42			
Error (c_2)	3,742.48	36	103.96			

* Low significance.
** High significance.

TABLE 10B. ANALYSIS OF VARIANCE OF DATA TAKEN AT 40 DAYS (TABLE 8B)

Source of variation	Sum of squares	Degrees of freedom	Mean square	F values		
				Found	Required	
					5 per cent	1 per cent
Total A × B	722.81	11				
Block	303.06	3	101.02	3.73	4.76	9.78
A	256.79	2	128.39	4.73	5.14	10.92
Error (a)	162.96	6	27.16			
Total A × S × L × B	18,463.80	47				
Less total A × B	17,740.99	36				
S	4,693.39	1	4,693.39	46.21**	4.21	7.88
A × S	388.99	2	194.49	1.91	3.35	5.49
L	7,666.46	1	7,666.46	75.48**		
A × L	268.82	2	134.41	1.32		
S × L	1,754.92	1	1,754.92	17.28**		
A × S × L	226.11	2	113.05	1.11		
Error (b)	2,742.30	27	101.57			
Total A × S × L × V × B	84,956.79	143				
Less total A × S × L × B	66,492.99	96				
V_1	167.75	1	165.75	6.95*	4.11	7.39
A × V_1	226.64	2	113.32	4.69*	3.26	5.25
S × V_1	.02	1	.02			
A × S × V_1	26.70	2	13.35			
L × V_1	40.42	1	40.42	1.68		
A × L × V_1	101.76	2	50.88	2.11		
S × L × V_1	2.77	1	2.77			
A × S × L × V_1	13.13	2	6.56			
Error (c_1)	868.41	36	24.12			
V_2	42,522.57	1	42,522.57	409.03**	4.11	7.39
A × V_2	266.85	2	133.42	1.28	3.26	5.25
S × V_2	6,400.52	1	6,400.52	61.57**		
A × S × V_2	483.69	2	241.84	2.33		
L × V_2	7,718.00	1	7,718.00	74.24**		
A × L × V_2	1,450.49	2	725.24	6.98**		
S × L × V_2	1,797.49	1	1,797.49	17.29**		
A × S × L × V_2	633.30	2	331.65	3.19		
Error (c_2)	3,742.48	36	103.96			

* Low significance.
** High significance.

of pots receiving uniform nutrient treatment across the bench. The design of the experiment was such as to necessitate the use of split-plot analyses as carried out under the discussion of Experiment A, in which it was also shown that, because variety variances were heterogeneous, variety comparisons could not be validly estimated by the pooled error variance. The data for each time of observation in Experiment B were also tested by Clark and Leonard's *(2)* modified chi-square test to ascertain homogeneity of variety variances. The results are as follows:

Time of observation	*X^2 of variety variances*
31 days	78.95
40 days	46.67
49 days	1.40

(Significant values of chi-square with two degrees of freedom are 5.99 at the 5 per cent level, and 9.21 at the 1 per cent level.)

The highly significant value of chi-square, 78.95, for variety variances in the data for the first count confirms that which was apparent from inspection of the raw data; *i. e.*, the preponderance of zero observations in Cook and Rowden varieties in contrast with the definite, but erratic, appearance of wilt symptoms in Half & Half plants indicated the marked distinctions in the distributions of the errors of observation between the two groups of varieties.

TABLE 10c. ANALYSIS OF VARIANCE OF DATA TAKEN AT 49 DAYS (TABLE 8c)

Source of variation	Sum of squares	De-grees of freedom	Mean square	F values		
				Found	Required	
					5 per cent	1 per cent
Total A × B	1,149.37	11				
Block	298.71	3	99.57	1.84	4.76	9.78
A	525.14	2	262.57	4.84	5.14	10.92
Error (a)	325.52	6	54.25			
Total A × S × L × B	30,749.82	47				
Less total A × B	29,600.45	36				
S	4,261.91	1	4,261.91	32.68**	4.21	7.88
A × S	1,254.40	2	627.20	4.81*	3.35	5.49
L	16,124.76	1	16,124.76	123.65**		
A × L	56.77	2	28.38			
S × L	4,296.81	1	4,296.81	32.95**		
A × S × L	83.11	2	42.55			
Error (b)	3,520.69	27	130.40			
Total A × S × L × V × B	142,119.71	142				
Less total A × S × L × B	111,369.89	96				
V_1	203.29	1	203.29	1.56	4.11	7.39
A × V_1	225.38	2	112.69		3.26	5.25
S × V_1	129.51	1	129.51			
A × S × V_1	263.62	2	131.80	1.01		
L × V_1	119.48	1	119.48			
A × L × V_1	274.54	2	137.27	1.05		
S × L × V_1	185.64	1	185.64	1.43		
A × S × L × V_1	462.03	2	231.03	1.77		
Error (c_1)	2,686.53	36	130.18			
V_2	88,364.58	1	88,364.58	977.70**	4.11	7.39
A × V_2	1,416.57	2	708.28	7.84**	3.26	5.25
S × V_2	1,512.04	1	1,512.04	16.73**		
A × S × V_2	1,991.73	2	995.86	11.02**		
L × V_2	3,518.90	1	3,518.90	38.93**		
A × L × V_2	1,955.96	2	977.98	10.82**		
S × L × V_2	700.31	1	700.31	7.74**		
A × S × L × V_2	2,106.01	2	1,053.01	11.65		
Error (c_2)	3,254.78	36	90.41			

* Low significance. ** High significance.

This same heterogeniety of variety variances is also evident from observations taken at 40 days after planting, but the most striking revelation of the chi-square tests is the disappearance of heterogeniety of error distributions between varieties at the third count. These tests show that the error variance for testing variety comparisons in the data for the first two counts should be segregated into two components: one for testing the comparison between Cook and Rowden varieties, V_1; and the other for testing the comparison of the mean of these two varieties with respective observations for Half & Half, V_2. Although variety variances were homogeneous at the third count, this same segregation of error variances was employed on the data for all counts in order to allow full comparisons of effects at different time intervals. In view of these considerations, the analyses in Tables 10a, 10b, and 10c were carried out in the same manner as that for the data of Experiment A in Tables 4 and 5.

The results of the analysis in Table 10a merely substantiates many of the conclusions that could have been drawn from the mean summaries of Table 9a. At the first count both sources and levels of nitrogen supply have a highly significant influence upon the development of wilt, there is no significant difference in response between Cook and Rowden varieties, and the differential in response between Half & Half and the other two varieties is highly significant. A consideration of the status of the raw data must be taken into account in interpreting the pooled-variety nutrient effects. Since there were but few observations other than zero for Cook and Rowden varieties, the results for nutrient effects shown in Table 10a are essentially those for Half & Half.

It was suggested from observation of the mean values for acidity levels in Table 9a that acidity may have had a significant influence upon the development of wilt up to this time. The variance ratio for effect A in Table 10a attains the 5 per cent level of significance, indicating that wilt development was conditioned by the pH of the nutrient supply. The significance of this factor might have been higher had not the nature of the experimental design, confounding of pH levels with subblocks, induced a loss of precision for testing acidity effects. The interrelationship of factors, $A \times S$ and $S \times L$, were conditioning disease intensity to a significant degree at this time. No nutritional effect related with the V_1 comparison was of significance, whereas several nutritional effects related to the V_2 comparison were significant. The significance found for interactions, $A \times V_2$ and

$A \times S \times V_2$, indicates that the magnitude of the differential in disease intensity between varietal groups, $(H \& H \text{ vs. } \frac{C - R}{2})$, was influenced by the acidity of the nutrient solution and by the source of nitrogen at a given acidity level. The high significance found for interactions, $L \times V_2$ and $S \times L \times V_2$, show the magnitude of the differences in response between varietal groups to be definitely influenced by the level of nitrogen supply, and the source of nitrogen within a given level of supply. Many of these important relationships between variety comparisons and nutrition upon disease development were not necessarily apparent in the original data.

The analysis in Table 10b upon the data taken at 40 days after planting presents a somewhat similar picture of the results to that shown by Table 10a, but with several distinct differences. Effects S, L, and V_2 continue to be highly significant with their respective variance ratios being considerably higher than those found for the data taken at the first count. However, effects A and $A \times S$ do not make a significant contribution to variability at the second count, showing that disease development during the 9-day interval obliterated the initially observed effects of acidity levels and $A \times S$ interaction. The importance of $S \times L$ interaction became more pronounced during the interval by fact that its variance ratio exceeded that required for high significance at the second count.

The comparison between Cook and Rowden varieties, V_1, and the effect of acidity levels upon this comparison, $A \times V_1$, were found to be significant contributors at the second count, whereas they lacked significance at the time of the first count. The changes in significance brought about during the 9-day interval within the V_2 comparisons are remarkable. The respective variance ratios, reproduced for comparative purposes, are:

	Variance ratios at:	
Effect	*First count*	*Second count*
V_2	55.71**	409.33**
$A \times V_2$	3.53*	1.28
$S \times V_2$	3.43	61.57**
$A \times S \times V_2$	4.03*	2.33
$L \times V_2$	10.39**	74.24**
$A \times L \times V_2$		6.98**
$S \times L \times V_2$	8.39**	17.29**
$A \times S \times L \times V_2$		3.19

* Low significance. ** High significance.

The initially observed influence of effects, A × V_2 and A × S × V_2, was obliterated during the interval between these two counts, while the influence of other nutritional effects upon the V_2 comparison was extensively amplified. However, for reasons other than pointing out that the time factor has definitely influenced the role of nutrients in conditioning host susceptibility, it would be premature to draw detailed conclusions from the variance ratios without consideration of those shown by the analysis in Table 10c for the data taken at 49 days after planting.

Wilt development during the second 9-day interval produced only a minor change in the significance of nutritional effects—interaction A × S attained significance. None of the effects involving V_1 comparison were significant at the final count, whereas every effect involving V_2 comparison was highly significant. In other words, at the termination of the experiment there was no significant difference in disease intensity between Cook and Rowden plants, as groups, and no disparity in this differential, (C *vs.* R), induced by nutritional effects, attained significant proportions. By contrast, the difference in wilt development between Half & Half and the other two varieties was not only extreme, but the disparities in the V_2 differences induced by nutritional effects were highly significant for every interrelationship of nutritional effects and V_2 comparison.

From this discussion it becomes evident that interpretations of disease development in Experiment B could not be dissociated from the time at which observations were made. Although parallel analyses of data taken at these different times of observations definitely reveal the importance of the time factor in studying the interrelationships, they do not permit a statistical evaluation of the relative importance of time in inducing modifications within other effects. The question immediately arises as to the correct procedure for taking into account the time factor. It should be recalled that the 432 observations presented were obtained by taking three observations on each of the 144 pots of plants, and do not represent 432 fully independent observations. Furthermore, it should be emphasized that the *experimental error* can only be affected by the size of the experiment, *i. e.*, by discrepancies among the 144 pots of plants; whereas the *sampling error* deals with discrepancies among the respective counts, *i. e.*, it is derived from the 432 observations on these 144 pots of plants. The foregoing analyses only involved an experimental error inasmuch as each set of observations was analyzed independently.

In the studies in which all observations are considered in the analysis it is essential to differentiate between the experimental error derived from the pooled observations and the sampling error that involved observational differences. This point is illustrated in Table 12.

In order to better illustrate the problem of interdependence of observational (time) effects, let the observations taken at 31 days be designated as t_1; then those at 40 days may be designated as equivalent to $(t_1 + t_2)$ with the increment t_2 representing the increase in disease intensity between the two times of observation. Similarly, the observations at 49 days may be represented as $(t_1 + t_2 + t_3)$, with t_3 representing the increment between the observations at 40 and 49 days. It now follows that if all of the observations in Tables 7a, 7b, and 7c were included in the same analysis of variance, the comparison for time would include differences among the groups: t_1, $(t_1 + t_2)$, and $(t_1 + t_2 - t_3)$. Time effects would be taken out according to magnitude of increments t_2 and t_3. This in itself would not give rise to serious objections; however, the partial identity of group components removes complete independence of the groups. The observations for disease intensity at 40 days are not independent of those at 31 days, and neither are those taken at 49 days independent of the previous observations. This contingency would appear to militate against the conventional use of the analysis of variance in studying these data. However, an analysis of covariance between the data at 31 days and that at 49 days would remove variability from the latter data due to concurrent variability with the former data. Then the test of significance applied to the corrected mean squares for data taken at 49 days would bring out the importance of the time interval between the two dates of observation. Similarly, an analysis of covariance between the means of the respective observations for 31 and 49 days and the observations for 40 days would remove variability from the latter data due to concurrent variability within the means of the respective observations at the former two dates. Comparing the medial set of observations with the mean of those at the extremes determines the linearity of the time effect; for if time effect is linear, the medial observation will exactly coincide with the mean of the two respective extremes. The test of significance applied to the corrected medial observations determines whether the observations at 40 days departed significantly from the linear response as set up by the data for 31 and 49 days.

TABLE 11. ANALYSES OF VARIANCE OF DATA FOR HALF & HALF VARIETY FOR EACH TIME OF OBSERVATION

Effect	D.F.	Mean square for data at:		
		31 days	40 days	49 days
Blocks	3	29.02	287.00	27.87
Source of N	1	445.30**	10,998.94**	4,822.07**
Level of N	1	643.33**	14,953.08**	11,822.75**
S × L	1	228.20*	3,157.80**	3,534.62**
Level of acidity	2	237.78*	178.12	934.13**
A × S	2	235.97*	103.67	1,214.23**
A × L	2	7.16	811.23*	768.53**
A × S × L	2	158.67	411.03	869.70**
Error	33	63.58	157.85	143.74

* Low significance.
** High significance.

Table 11 presents the straightforward analyses of variance applied to only the data for Half & Half variety for each of the three times of observation.

The main effects of sources and levels, and their interaction, are relatively consistent for the analyses at all three recordings. All other effects and interactions vary considerably in attained significance, dependent on the time at which the observations were made. Attention is thus focused on the necessity for studying the interaction of time with the nutrient effects in order to comprehend completely the responses of this variety as measured by disease susceptibility. Table 12 presents the results of taking out the linear and curvilinear effects of time in the Half & Half data by covariance, as compared with the results of an analysis of variance, in which time is measured as one of the component factors.[5] The results are essentially the same whether the T_1 (30 *vs.* 49 days) and T_2 ($31 + 49$ *vs.* 2×40 days) comparisons are studied by variance or covariance. The only discrepancy is that the S × L interaction with the T_2 effect was barely significant by variance and not quite significant by covariance. A study of the basic implications of these two procedures reveals that there should be no difference in the results by these two procedures, since covariance measures the significance of variability in observations taken at one count after due allowance has been made for concomitant variability in observations at the other count; and similarly, variance measures the significance in the disparities in the differentials between respective means derived at

[5] Linear effects of time refers to differences between the two extremes, and the curvilinear effects of time refers to differences between the medial observations and the means of the respective extremes.

the different times of observation. Although the findings presented in Table 12 appear to indicate that the lack of independence of the times of observation is not a serious objection to the use of the analysis of variance for studying a series of observations on the same group of plants, there are other aspects to this data inherent in the lack of independence which would make for rather abstruse interpretations.

The mean squares for the pooled time values for each nutrient effect (Table 12) was included to make the analysis of the data for the Half & Half variety complete. It may be recalled that the observations at 31 days were designated as t_1, those at 40 days $(t_1 + t_2)$, and those at 49 days $(t_1 + t_2 + t_3)$. It now becomes evident that the nutrient effects ascertained by pooling three times of observation (essential in the straightforward analysis of the pooled data) is the sum of the above three time designations for each effect: $(t_1) + (t_1 + t_2) + (t_1 + t_2 + t_3)$, or $3\,t_1 + 2\,t_2 + t_3$. In other words, the procedure has resulted in unevenly weighting disease development at different periods. Thus, in

TABLE 12. COMPARISON OF ANALYSIS OF VARIANCE AND ANALYSIS OF COVARIANCE FOR TAKING OUT TIME EFFECTS OF DATA FOR HALF & HALF VARIETY (TABLE 7)

		Mean squares				
		Analyses of variance			Analyses of covariance [1]	
Effect	D.F.	Three times of observations pooled	Interactions with T_1 (31 days vs. 49 days)	Interactions with T_2 $\left(\frac{31 + 49}{2}\right.$ vs. 40 days$\left.\right]$	T_1 comparisons (data for 49 days corrected for effects observed at 31 days)	T_2 comparisons (data for 40 days corrected for mean of effects observed at 31 and 49 days)
Blocks	3	225.62				
Time	1		72,572.50**	439.31**		
Source	1	12,729.48**	1,168.31**	2,368.44**	2,748.73**	2,032.00**
Level	1	24,198.39**	4,641.21**	1,581.56**	8,662.16**	814.31**
S × L	1	5,922.59**	986.24**	311.25*	2,457.66*	552.05*
Acidity	2	285.64	1,019.61**	33.09	116.09	54.10
A × S	2	1,444.65**	381.32**	28.35	86.39**	12.86
A × L	2	961.38*	457.67**	170.78	82.39**	27.81
A × S × L	3	1,252.98**	213.36**	3.63	55.11*	9.65
Error	33	211.79 [2]	65.44 [3]	72.55 [3]	133.80	109.96

* Low significance.
** High significance.
[1] Analysis of covariance carried out as described by Snedecor *(7)*.
[2] Experimental error.
[3] Sampling error.

Table 12 the net effect of nutrients is the result of taking three times the values for the first observed disease symptoms, twice times those developing during the next 9-day period, and only once times the values for those symptoms developing between the 40 to 49-day period. Such a procedure is not tenable since it necessarily makes the interpretation of the observed nutrient responses involved. Consequently, serial observations must be studied on the increment basis, which not only removes dependence in the time effect, and thereby obviating any objection to including this factor in the analysis of variance, but also permits total sum of increments to be synonymous with the final count that would be used for the net effect of nutrients or other factors exclusive of counts or the time factor.

Single degree of freedom analysis. It was pointed out in the discussion of the time factor that the two degrees of freedom associated with time could be partitioned into individuals; one for the comparison between wilt development during the 22 to 31-day period and the wilt development during the 40 to 49-day period, and the other for a comparison between the wilt development during the 31 to 40-day period and the mean of the other two periods.[6] As stated earlier, the first comparison denotes the linear effect of the time factor between the two extreme observations, and the second comparison designates the curvilinear (or quadratic) effect of the time factor, or the departure of the medial observation from the linear regression line between the two extremes. It follows that any effect associated with multiple degrees of freedom may be partitioned into individual comparisons. However, whenever the levels of a factor may not be designated by a numerical characterization, such as is the case with varieties, comparisons may be made on an arbitrary basis, *i. e.*, the two levels of varieties showing least disparity are taken out as the linear effect, and further comparisons between the mean of a primary group and another factor or group of factors are taken out as quadratic comparisons. This will be illustrated in the development of an experimental example.

The net arithmetical effect for a given factor or interaction may be expressed according to formal relationships. For example, in an experiment involving two sources of nitrogen, two levels of nitrogen supply, and three acidity levels, numerical summation

[6] Since no wilt symptoms were in evidence at 22 days after planting, the first series of observations at 31 days may be regarded as the first 9-day increment.

of a given effect would be in accordance with the expanded form of the following formal expressions:[7]

$$
\begin{aligned}
S &= (s_1 - s_2)\ (l_1 + l_2)\ (a_1 + a_2 + a_3)\\
L &= (s_1 + s_2)\ (l_1 - l_2)\ (a_1 + a_2 + a_3)\\
S \times L &= (s_1 - s_2)\ (l_1 - l_2)\ (a_1 + a_2 + a_3)\\
A_1 &= (s_1 + s_2)\ (l_1 + l_2)\ (a_1 - a_3)\\
A_1 \times S &= (s_1 - s_2)\ (l_1 + l_2)\ (a_1 - a_3)\\
A_1 \times L &= (s_1 + s_2)\ (l_1 - l_2)\ (a_1 - a_3)\\
A_1 \times S \times L &= (s_1 - s_2)\ (l_1 - l_2)\ (a_1 - a_3)\\
A_2 &= (s_1 + s_2)\ (l_1 + l_2)\ (a_1 - 2a_2 + a_3)\\
A_2 \times S &= (s_1 - s_2)\ (l_1 + l_2)\ (a_1 - 2a_2 + a_3)\\
A_2 \times L &= (s_1 + s_2)\ (l_1 - l_2)\ (a_1 - 2a_2 + a_3)\\
A_2 \times S \times L &= (s_1 - s_2)\ (l_1 - l_2)\ (a_1 - 2a_2 + a_3)
\end{aligned}
$$

The expansion of the first relationship of the above group becomes:

$$
\begin{aligned}
S = {} & s_1l_1a_1 + s_1l_1a_2 + s_1l_1a_3 + s_1l_2a_1 + s_1l_2a_2 + s_1l_2a_3 -\\
& s_2l_1a_1 - s_2l_1a_2 - s_2l_1a_3 - s_2l_2a_1 - s_2l_2a_2 - s_2l_2a_3
\end{aligned}
$$

Each term in this expression may be replaced by the numerical observation associated with a given combination of levels of all factors involved. Thus, in Table 13 the observed values for the Source-Level-Acidity combinations within Half & Half variety in Block I at the 49-day time of observation are presented as taken directly from Table 8c. Also, the treatment designations are given as derived from the above formal expression, or as may have been derived from the designation in Figure 2. Under the column headed "S" in Table 13 are the signs that accompany the various observed values for treatment effects, as given by the expansion of the formal expression for the effect "S." The other columns, headed by designations for the various component effects and interactions, show the signs to be used in summating the observed values in accordance with the formal expressions. The net numerical values for each summation is given in the bottom row of the column.

To complete a study of the above nutritional effects upon the wilt susceptibility of Half & Half variety at the final reading, the procedure indicated in Table 13 is also carried out for the observed values in blocks II, III, and IV. The calculation of the mean square for a given effect together with its individual error term is carried out as in Table 14, which illustrates this calculation

[7] S denotes net effect between sources of nitrogen; L, net effect between levels of nitrogen; A1, net effect between high and low levels of acidity; A2, net effect between medium levels and mean of high and low levels of acidity; and s_1, s_2, l_1, l_2, a_1, a_2, and a_3 denote respective levels of these respective effects.

TABLE 13. CALCULATION OF SUMMARIZED NUTRITIONAL EFFECTS WITHIN HALF & HALF VARIETY FOR OBSERVATIONS TAKEN AT 49 DAYS

Solution No.	Treatment	Observed value	Effects										
			S	L	SL	A_1	A_1S	A_1L	A_1SL	A_2	A_2S	A_2L	A_2SL
1	$s_1h_1a_1$	71.2	+	+	+	+	+	+	+	+	+	+	+
2	$s_1h_1a_2$	33.7	+	+	+					−2	−2	−2	−2
3	$s_1h_1a_3$	42.5	+	+	+	−	−	−	−	+	+	+	+
4	$s_1h_2a_1$	70.0	+	−	−	+	+	−	−	+	+	−	−
5	$s_1h_2a_2$	75.0	+	−	−					−2	−2	2	2
6	$s_1h_2a_3$	58.7	+	−	−	−	−	+	+	+	+	−	−
7	$s_2h_1a_1$	23.7	−	+	−	+	−	+	−	+	−	+	−
8	$s_2h_1a_2$	57.5	−	+	−					−2	2	−2	2
9	$s_2h_1a_3$	45.0	−	+	−	−	+	−	+	+	−	+	−
10	$s_2h_2a_1$	100.0	−	−	+	+	−	−	+	+	−	−	+
11	$s_2h_2a_2$	100.0	−	−	+					−2	2	2	−2
12	$s_2h_2a_3$	100.0	−	−	+	−	+	+	−	+	−	−	+
Sums		777.3	−75.1	−230.1	117.5	18.7	61.3	−3.9	38.7	−21.3	71.9	21.3	13.9

for S effect: the value 75.1 for Block 1 in Table 14 being taken directly from the bottom of the "S" column in Table 13. Similar values in Table 14 are similarly derived. The error term for testing S effect will consist of the value for the three degrees of freedom associated with Source × Block interaction. The partitioning of these three degrees of freedom is also given in Table 14.

TABLE 14. CALCULATION OF MEAN SQUARE FOR AN EFFECT TOGETHER WITH ITS INDIVIDUAL ERROR TERMS

Components of variance	Block I	Block II	Block III	Block IV	Sums	k	$\frac{\text{Sums}^2}{k}$ (mean square)
S effects	−75.1	−123.7	−203.8	−78.5			
Total	+	+	+	+	−481.1	48	4,822.03
Error: $S \times B_1$	+	—			48.6	24	98.41
Error: $S \times B_2$	+	+	−2		208.8	72	605.52
Error: $S \times B_3$	+	+	+	−3	−167.1	144	193.91

$$F = \frac{4,822.03}{299.28} = 16.11$$

Mean error = 299.28

That is, one degree of freedom for Source × Block interaction ($S \times B_1$) is due to the difference between S effects in blocks I and II; another, ($S \times B_2$), is due to the difference between S effect in block III and the mean of the effects for blocks I and II; and the third, ($S \times B_3$), is due to the difference between S effect in block IV and the mean for the other three blocks. The mean square for S effect: $\frac{(\Sigma \text{ S differences})^2}{k} = \frac{(-481.1)^2}{48} = 4,822.03$. The k value in this formulation is derived from the number of observations involved in each unit (blocks in this example) and the square of the number of times each unit is considered. Hence, since there are 12 observed values represented by each unit (Table 13), and each unit is taken only once $k = (1^2 + 1^2 + 1 + 1)12 = 48$ for S effect. For the components of error for S, the k values are as follows:

For $S \times B_1$, $k = (1^2 + 1^2)12 = 24$
$S \times B_2$, $k = (1^2 + 1^2 + 2^2)12 = 72$
$S \times B_3$, $k = (1^2 + 1^2 + 1^2 + 3^2)12 = 144$

The mean square for each S × B interaction is calculated in the same manner as the mean square for S effect. These three components of the error for S are pooled and since three degrees of

freedom are represented, their mean is the mean square for testing S effect, as shown by the relationship at the bottom of Table 14.

The mean square for S effect for data used in the above example would be identical if it had been calculated by the methods used for the analysis of Experiment A:

$$\frac{(1{,}276.0)^2 + (1{,}757.1)^2}{24} - \frac{(3{,}033.1)^2}{48} = 4{,}822.03$$

Although this procedure is less involved for the calculation of the mean square of a main effect than the one just discussed, it lacks any means of calculating the specific error term for a given effect.

The modification in the calculation of the mean square for the individual degree of freedom of a factor studied at more than two levels follows: If the example used in Table 14 had involved either effect A_1 or A_2, the only modification in procedure would have been in the k values employed. Thus, only eight values are used from each block (Table 13) for the calculation of A_1 effect in the example. In the relationships just given for the calculation of k values for studying S, the multiplier would be changed from 12 to 8 for deriving k values with which to study A_1.

The derivation of k values for studying A_2 (the curvilinear or quadratic effect of A) is more involved. All 12 observations in a block (Table 13) are utilized, but four of them, the intermediate levels of the effect A, are taken twice. Hence:

For A_2,

$$k^8 = [(1^2 + 2^2 + 1^2) + (1^2 + 2^2 + 1^2) + (1^2 + 2^2 + 1^2) + (1^2 + 2^2 + 1^2)]4 = (1^2 + 2^2 + 1^2)\ (4 \times 4) = 96$$

For A_2B_1,

$$k = [(1^2 + 2^2 + 1^2)\ (1^2 + 2^2 + 1^2)]4 = (1^2 + 2^2 + 1^2)\ (2 \times 4) = 48$$

For A_2B_2,

$$k = [(1^2 + 2^2 + 1^2)\ (1^2 + 2^2 + 1^2)\ (2^2 + 4^2 + 2^2)]4 = (1^2 + 2^2 + 1^2)\ [(2 + 2^2)4] = 144$$

For A_2B_3,

$$k = [(1^2 + 2^2 + 1^2) + (1^2 + 2^2 + 1^2) + (1^2 + 2^2 + 1^2) + (3^2 + 6^2 + 3^2)]4 = (1^2 + 2^2 + 1^2)\ (3 + 3^2)4 = 288$$

Although this formulation may look formidable for the obtaining of a set of factors used in calculating a given effect, a little experience in using these factors will enable one to get a better insight into certain of their basic relationships, and permits one to write in the factors without giving thought to the derivation.

The foregoing example was uncomplicated by the variety and time effects inherent in the composite data in order that the

[8] This formulation is sometimes designated as nk. The value, 4, is used since each form, $(1^2 + 2^2 + 1^2)$, is taken four times in each block.

presentation of the method be made as simple as possible. The incorporation of variety effects in the consideration of the data may be illustrated by aid of Table 15. The values under "Half & Half" were taken directly from the base of Table 13; the values under "Cook" and "Rowden" were calculated in the same manner as those for Half & Half. The "sum of varieties" is the summation of the respective nutrient effects for all varieties. These values are used in calculation of the pooled variety-nutrient effects as shown in Table 14 (with the use of the correct k values). Comparisons between Cook and Rowden and their interactions with nutrient effects are given under "V_1 comparisons." The value, 46.4, in the "sums" series under "V_1 comparisons" is employed in the calculation of V_1 effect *per se*. Comparisons between Half & Half and the mean of Cook and Rowden, together with their respective interactions, are given under "V_2 comparisons." The values involving varieties and their nutrient interactions are assembled with similar data from blocks II, III, and IV, and used in the calculations of the various mean squares and error terms, according to the procedure of Table 14.

With the incorporation of varieties into the study, the k values must be suitably modified. Thus, the calculations for the k values are:

For S,

$$k = (1^2 + 1^2 + 1^2 + 1^2)36 = 144$$

For V_1,

$$k = (1^2 + 1^2 + 1^2 + 1^2)24 = 96$$

For V_2,

$$k = [(1^2 + 2^2 + 1^2) + (1^2 + 2^2 + 1^2) + (1^2 + 2^2 + 1^2) + (1^2 + 2^2 + 1^2)]12 = (1^2 + 2^2 + 1^2)48 = 288$$

For V_1A_1,

$$k = (1^2 + 1^2 + 1^2 + 1^2)16 = 64$$

For V_2A_1,

$$k = [(1^2 + 2^2 + 1^2) + (1^2 + 2^2 + 1^2) + (1^2 + 2^2 + 1^2) + (1^2 + 2^2 + 1^2)]8 = (1^2 + 2^2 + 1^2)32 = 192$$

For V_2A_2, (modification of k from V_2)

$$k = (1^2 + 2^2 + 1^2)48/3\ (1^2 + 2^2 + 1^2) = (1^2 + 2^2 + 1^2)96 = 576$$

In the latter relationship the second quadratic form for A_2 is substituted for the three values for A levels used when values for this factor are pooled. Since the inclusion of a two-level effect in an interaction does not modify the k value, the above formulations illustrate the derivations of all k values for nutrient-variety effects. The k values to be used for the errors are merely modifications of the above relationships, according to the procedure

TABLE 15. ILLUSTRATION OF CALCULATION OF V_1 AND V_2 COMPARISONS AS WELL AS POOLED VARIETY EFFECTS [1]

Effect	Nutrient effects for varieties			Sum of varieties	V_1 comparisons	V_2 comparisons
	Cook [2]	Rowden [2]	Half & Half			
Sums	138.4	184.8	777.3	1,100.5	— 46.4	—1,231.4
S	— 28.6	— 30.0	— 75.1	—133.7	1.4	91.6
L	—113.4	— 87.6	—230.1	—431.1	— 25.8	259.2
S × L	53.6	— 22.4	117.5	148.7	76.0	— 203.8
A_1	2.8	— 32.6	18.7	— 11.1	35.4	— 67.2
A_1 × S	— 55.2	7.4	61.3	13.5	— 62.6	— 170.4
A_1 × L	22.2	60.0	— 3.9	78.3	— 37.8	90.0
A_1 × S × L	80.2	— 50.0	38.7	68.9	—130.0	— 47.2
A_2	14.8	— 25.2	— 21.3	— 31.7	40.0	32.2
A_2 × S	72.8	30.0	71.3	174.1	42.8	— 39.8
A_2 × L	10.2	148.2	21.3	179.7	—138.0	115.8
A_2 × S × L	— 47.8	— 7.4	113.9	58.7	— 40.4	— 283.0

[1] Data derived from Block I of Table 7c, according to method of Table 13.
[2] Cook 307; Rowden 2088.

previously illustrated. However, by way of example, Table 16 presents the calculation of the mean square for the effect $V_2 \times A_2 \times S$ together with its individual error term.

Segregating the data, according to time effects, merely involves an elaboration of the foregoing procedure. As already pointed out, the time factor must be studied on the increment basis. Consequently, the data in Table 8a may be taken as that for the first time level; the increments between the respective observations in Table 8a and 8b as the second time level; and the increments between the respective observations in Table 8b and 8c as the third time level. Accordingly, the data in Table 8c presents the variety-nutrient observations with time effects pooled, since the observations at the final reading must be the sum of the increments. Hence, the data used in Tables 15 and 16 are identical

TABLE 16. ILLUSTRATION OF CALCULATIONS OF MEAN SQUARE FOR EFFECTS $V_2 \times A_2 \times S$ TOGETHER WITH ITS INDIVIDUAL ERROR TERM

Components of variance	Block I	Block II	Block III	Block IV	Sums	k	Sums [2] / k (mean square)
$V_2 \times A_2 \times S$ effect	—39.8	—3.0	32.9	138.8			
Total	+	+	+	+	128.9	576	28.85
Error $V_2 \times A_2 \times S \times B_1$	+	—			— 36.8	288	4.70
Error $V_2 \times A_2 \times S \times B_2$	+	+	—2		—108.6	864	13.65
Error $V_2 \times A_2 \times S \times B_3$	+	+	+	—3	—426.3	1,728	105.17

Mean error = 41.19

with those for the pooled effect of the three time levels. However, since each observation in the data used for illustration is the sum of three observations from the composite data, the k values in the construction tables must be suitably modified. This may be illustrated by the construction tables of Table 17, which were taken from actual tables used in computing the data of Table 18. In this experiment, as in Experiment A, T_1 is taken as the comparison between the first observation and the increments between the second and third observations (first *vs.* final increments). T_2 is taken as the comparison of the increments between the first and second observations and the mean of the two increments used in T_1 comparisons, and shows whether or not development of disease intensity was linear over the period of observation.

One of the values of a single degree of freedom analysis lies with the fact that it is possible to make a direct study of the contribution of each degree of freedom associated with a multiple factor or interaction. While the indirect method (calculating the

TABLE 17. SAMPLE CONSTRUCTION TABLE USED IN CALCULATING DATA OF TABLE 18

Effect		Block I	Block II	Block III	Block IV	Sums	k	Mean square
S effect		133.7	217.2	316.3	116.2	783.4	432	1,420.64
Error	+	—			— 83.5	216	32.28	
	+	+	—2		— 281.7	648	122.17	
	+	+	+	—3	318.6	1,296	78.32	
							Mean error =	77.69
T_1 effect		—369.6	—326.1	—350.2	—406.2	—1,452.1	288	7,321.51
Error	+	—			— 43.5	144	13.14	
	+	+	—2		4.7	432	.05	
	+	+	+	—3	172.7	864	34.52	
							Mean error =	15.90
T_2 effect		—456.2	—311.3	—150.4	—264.6	—1,182.5	864	1,618.41
Error	+	—			— 144.9	432	48.50	
	+	+	—2		— 466.7	1,296	168.06	
	+	+	+	—3	— 124.1	2,592	5.94	
							Mean error =	74.20
$T_2 \times V_2 \times A_2 \times S \times L$		—325.6	145.1	325.3	— 11.9	132.9	3,456	5.12
Error	+	—			— 470.7	1,728	128.22	
	+	+	—2		— 831.1	5,184	133.24	
	+	+	+	—3	180.5	10,368	3.14	
							Mean error =	88.20

TABLE 18. ANALYSIS OF DATA OF TABLE 8 ACCORDING TO METHOD OF INDIVIDUAL DEGREES OF FREEDOM WITH INDIVIDUAL ERROR TERMS

Effect	Mean square	Mean error	F value	Significance with pooled error	
				Homologous groups	Completely pooled[1]
S	1,420.64	77.69	18.30*	**	**
L	5,374.92	14.04	282.82**	**	**
S × L	1,432.25	24.27	59.01**	**	**
A_1	19.58	24.43			
$A_1 \times S$	131.89	41.63	3.17		
$A_1 \times L$	16.20	93.11			
$A_1 \times S \times L$	2.47	25.28			
A_2	155.46	11.74	13.24*		*
$A_2 \times S$	286.23	62.83	4.56	**	**
$A_2 \times L$	2.72	46.51			
$A_2 \times S \times L$	26.56	3.39	7.83		
Mean error (a)		38.62			
V_1	67.76	28.97	2.34		
$V_1 \times S$	43.17	9.53	4.53		
$V_1 \times L$	39.83	12.89	3.09		
$V_1 \times S \times L$	61.88	38.79	1.59		
$V_1 \times A_1$	75.12	10.83	6.94		
$V_1 \times A_1 \times S$	79.44	54.91	1.45		
$V_1 \times A_1 \times L$	81.51	14.71	5.54		
$V_1 \times A_1 \times S \times L$	130.84	149.12			
$V_1 \times A_2$	.03	21.00			
$V_1 \times A_2 \times S$	8.43	83.31			
$V_1 \times A_2 \times L$	10.00	60.98			
$V_1 \times A_2 \times S \times L$	23.16	42.68			
Mean error (b)		43.98			
V_2	29,454.86	6.59	4,469.62**	**	**
$V_2 \times S$	504.01	37.68	13.38*	**	**
$V_2 \times L$	1,172.97	36.91	31.78*	**	**
$V_2 \times S \times L$	233.44	52.35	4.46	**	*
$V_2 \times A_1$	214.50	6.44	33.31*	*	*
$V_2 \times A_1 \times S$	654.29	66.25	9.88	**	**
$V_2 \times A_1 \times L$	520.03	12.49	41.64**	**	**
$V_2 \times A_1 \times S \times L$	117.45	40.28	2.91		
$V_2 \times A_2$	257.69	48.23	5.34	*	**
$V_2 \times A_2 \times S$	9.61	13.73			
$V_2 \times A_2 \times L$	130.95	12.89	10.16*		
$V_2 \times A_2 \times S \times L$	585.55	17.69	33.10*	**	**
Mean error (c)		29.29			
Mean experimental error		37.26			
T_1	7,321.50	15.90	460.47**	**	**
$T_1 \times S$	156.49	56.70	2.76		
$T_1 \times L$	252.56	12.84	19.67*	*	
$T_1 \times S \times L$	88.55	14.48	6.11		
$T_1 \times A_1$	477.54	19.02	25.11*	**	**
$T_1 \times A_1 \times S$	25.67	127.26			
$T_1 \times A_1 \times L$	5.47	26.15			
$T_1 \times A_1 \times S \times L$	160.97	22.85	7.04		
$T_1 \times A_2$	194.14	22.24	8.72		
$T_1 \times A_2 \times S$	322.50	99.44	3.24	**	*
$T_1 \times A_2 \times L$	82.81	6.58	12.58*		
$T_1 \times A_2 \times S \times L$	60.19	70.68			
Mean error (d)		41.18			
$T_1 \times V_1$	.0008	24.43			
$T_1 \times V_1 \times S$	69.12	4.85	14.25*		
$T_1 \times V_1 \times L$	158.41	25.72	6.16		
$T_1 \times V_1 \times S \times L$	68.64	29.05	2.36		
$T_1 \times V_1 \times A_1$	4.46	16.24			
$T_1 \times V_1 \times A_1 \times S$	60.91	57.60	1.06		
$T_1 \times V_1 \times A_1 \times L$	6.17	40.78			
$T_1 \times V_1 \times A_1 \times S \times L$	113.81	169.51			
$T_1 \times V_1 \times A_2$	.14	98.31			
$T_1 \times V_1 \times A_2 \times S$	50.68	94.67			
$T_1 \times V_1 \times A_2 \times L$	12.87	53.03			
$T_1 \times V_1 \times A_2 \times S \times L$	45.86	24.86	1.85		
Mean error (e)		47.41			

— Continued.

TABLE 18. ANALYSIS OF DATA OF TABLE 8 ACCORDING TO METHOD OF INDIVIDUAL DEGREES OF FREEDOM WITH INDIVIDUAL ERROR TERMS—*(Continued)*

Effect	Mean square	Mean error	F value	Significance with pooled error	
				Homologous groups	Completely pooled[1]
$T_1 \times V_2$	1,091.20	148.33	7.36	**	**
$T_1 \times V_2 \times S$	1,609.35	65.17	24.69*	**	**
$T_1 \times V_2 \times L$	1,135.69	96.96	11.71*	**	**
$T_1 \times V_2 \times S \times L$	365.45	46.77	7.81	*	*
$T_1 \times V_2 \times A_1$	522.90	24.22	21.59*	*	**
$T_1 \times V_2 \times A_1 \times S$	35.10	6.78	5.18		
$T_1 \times V_2 \times A_1 \times L$	18.24	[illegible]			
$T_1 \times V_2 \times A_1 \times S \times L$	4.66	24.32			
$T_1 \times V_2 \times A_2$	243.19	41.36	5.87		
$T_1 \times V_2 \times A_2 \times S$	.58	[illegible]			
$T_1 \times V_2 \times A_2 \times L$	26.37	9.79	2.69		
$T_1 \times V_2 \times A_2 \times S \times L$	52.23	37.89	1.38		
Mean error (f)		[illegible]			
T_2	1,618.41	74.20	21.81*	**	**
$T_2 \times S$	1,563.05	110.40	14.10*	**	**
$T_2 \times L$	1,199.68	88.68	13.53*	**	**
$T_2 \times S \times L$	140.89	34.94	4.03		
$T_2 \times A_1$	14.00	47.32			
$T_2 \times A_1 \times S$	7.20	155.25			
$T_2 \times A_1 \times L$	241.54	48.62	4.97		
$T_2 \times A_1 \times S \times L$	1.69	24.23			
$T_2 \times A_2$	232.17	73.81	3.14		
$T_2 \times A_2 \times S$	152.18	159.15			
$T_2 \times A_2 \times L$	209.31	58.54	3.57		
$T_2 \times A_2 \times S \times L$	25.53	128.58			
Mean error (g)		83.64			
$T_2 \times V_1$	72.10	32.38	2.23		
$T_2 \times V_1 \times S$	14.89	6.10	2.44		
$T_2 \times V_1 \times L$	135.14	22.04	6.13	*	
$T_2 \times V_1 \times S \times L$	14.63	7.51	1.95		
$T_2 \times V_1 \times A_1$	74.99	1.22	61.47**		
$T_2 \times V_1 \times A_1 \times S$	34.86	21.43	1.63		
$T_2 \times V_1 \times A_1 \times L$	12.08	15.54			
$T_2 \times V_1 \times A_1 \times S \times L$	25.27	67.26			
$T_2 \times V_1 \times A_2$	.91	42.04			
$T_2 \times V_1 \times A_2 \times S$	51.05	53.26			
$T_2 \times V_1 \times A_2 \times L$	7.95	24.94			
$T_2 \times V_1 \times A_2 \times S \times L$	6.43	18.08			
Mean error (h)		25.98			
$T_2 \times V_2$	5,913.34	152.08	38.88**	**	**
$T_2 \times V_2 \times S$	3,966.99	144.76	27.42*	**	**
$T_2 \times V_2 \times L$	3,594.36	185.59	19.37*	**	**
$T_2 \times V_2 \times S \times L$	757.63	78.01	9.71	*	**
$T_2 \times V_2 \times A_1$	142.88	43.11	3.31		
$T_2 \times V_2 \times A_1 \times S$	74.78	126.82			
$T_2 \times V_2 \times A_1 \times L$	808.86	41.41	19.53*	*	**
$T_2 \times V_2 \times A_1 \times S \times L$	100.29	74.11	1.35		
$T_2 \times V_2 \times A_2$	367.71	209.58	1.75		*
$T_2 \times V_2 \times A_2 \times S$	285.60	276.10	1.03		*
$T_2 \times V_2 \times A_2 \times L$	189.05	143.75	1.31		
$T_2 \times V_2 \times A_2 \times S \times L$	5.12	88.20			
Mean error (i)		130.29			
Mean sampling error		68.51			

* Low significance.
** High significance.
[1] Experimental errors are pooled, and sampling errors are pooled.

difference required for significance) is equally applicable to a study of main effects, no such convenience exists for measuring the component variances of a multiple degree interaction. The information can be gained from the multiple degree analysis, but it is an awkward means of avoiding single degree analysis. As an illustration of the segregation of the individual components of a multiple degree factor, it may be seen from Table 18 that one degree of freedom associated with variety effects, V_1, has a mean square of only 67.76 and is not significant; the other degree of freedom, V_2, has a mean square of 29,454.86, well beyond the requirement for high significance. On the pooled basis the variety effect would be highly significant, but it would not divulge the information that the comparison between Cook and Rowden is of no significance, and that practically all of the large mean square associated with varieties is due to the comparison between Half & Half and the mean of the other two varieties. Similarly, the interaction between varieties and acidity levels would not be significant on the multiple degree of freedom basis, but when components of this interaction are stratified into their individual effects, one of them, $V_1 \times A_2$, contributes practically nothing, 0.03, to the aggregate effect variance; another, $V_1 \times A_1$, is not significant; and the other two, $V_2 \times A_1$ and $V_2 \times A_2$, have mean squares that are significant or approach significance. From actual study of the components of a given effect or interaction, one can arrive at a high degree of precision in the segregation of significant contributors in an experiment—an undoubted advantage over the consideration of multiple degree factors in their composite state.

It is evident from Table 18 that there is a large range in individual error variances, 1.22 to 276.10. That is, the variance for each effect is tested by an error variance commensurate with the precision with which this effect was measured. For example, the effect, $T_2 \times V_1 \times A_1$, has a variance of only 74.99, yet it is highly significant because its respective error variance is only 1.22. On the other hand, the effect, $T_2 \times V_2 \times S \times L$, with a variance of 757.63, is not significant because its respective error variance is 78.01. Although the pooled error term masks the precision with which a given effect may or may not be tested, it does obviate the disadvantage of individual error terms inherent in experiments with a small number of replicates; *i. e.*, the present experiment with only four replicates permits only three degrees

of freedom for individual error terms, and requires variance ratios of 10.13 and 34.12 for low and high significance, respectively.

The above disadvantage may be overcome by pooling all the errors of observation to form a pooled mean square for measuring each of the individual effects. However, this is deemed advisable only when the individual error variances may be considered to be members of the same homogeneous population. In the present instance, a test of homogeniety by a modified chi-square, as used by Brandt *(1)*, and by Clark and Leonard *(2)*, reveals that the individual error variances in this experiment may be regarded as a homogeneous group. Consequently, the measurements of significance by the fully pooled error may be compared with the resultant significance as derived by testing with individual errors. The degree of significance of the various effects when measured by the fully pooled error is presented in Table 18.

Many effects are significant whether they are tested by their respective individual error or by the fully pooled error, leaving little doubt as to the importance of these effects. The lack of consistency in the significance of certain effects by the two methods of testing would appear to lead to confusion in the interpretations; but either method may lead to a biased estimate, depending on the data being studied. Use of the pooled error may result in a questionable piling up of degrees of freedom for testing single degree effects, even though errors are homogeneous, due to the fact that certain effects are derived by use of only a portion of the whole data. The use of the errors of observation over the whole data as a means for testing the reliability of effects involving only a portion of the data may be open to question, *e. g.*, effect $T_1 \cdot V_1 \cdot A_1$ is derived by use of only 8 27 of the 432 observations (actually 128 observations), yet the pooled error term involves 216 degrees of freedom. Obviously, such a contingency could lead to a serious bias in the interpretations. On the other hand, the use of individual error terms does not take into account the total number of observations employed in measuring a given effect and may unduly jeopardize the validity of the associated variance ratio.

A method to cope with such difficulties as those just cited has been suggested *(9)*. It involves the grouping of the individual error variances according to groups of homologous effects and results in an appreciable increase in degrees of freedom associated with error variance without the introduction of the grounds

for objection evident for the use of the completely pooled error term. This was accomplished in the present case by grouping the error variances for nutritional relationships within each category of time and variety comparisons. Thus, in Table 18 effects S through $A_2 \times S \times L$ may be measured by error (a), (38.62); effects V_1 through $V_1 \times A_2 \times S \times L$ may be measured by error (b), (43.98); etc. Although the three error terms associated with the groups within the pooled effect of time, errors (a), (b), and (c), show but little disparity; those associated with the quadratic effect of time, errors (g), (h), and (i), vary from 25.98 to 130.29 and are definitely heterogeneous. Consequently, the stratification of errors according to homologous effects not only permits a feasible number of degrees of freedom to be available for error variance, but also takes into account the heterogeniety in the precision of measuring the various categories of effects. However, it must be emphasized that, although this method of regrouping errors appears to have merit, it should not be used without due caution, inasmuch as statisticians have presented little information upon the basic implications and limitations of this procedure.

In Table 18 significance of effects as determined by errors of homologous groups follows rather closely the significance as ascertained by the pooled error term. The discrepancies that do exist are mostly due to an apparent overestimate of significance by the pooled error. On the other hand, in comparison with the results obtained by using the regrouped errors, the individual error terms appear, for the most part, to give an underestimate of effects. Consequently, such interpretations as may be given to the results of this experiment may be most logically based upon the findings resulting from the use of the regrouped errors.

In brief, the results of Experiment B, as derived from Table 18, may be summarized as follows: The intensity of disease development in this experiment was very definitely conditioned by the source of nitrogen employed, by the level of nitrogen supplied, and by the interrelationship between these factors. The acidity of the substrate had little effect upon wilt development, but it does appear that the influence of sources of nitrogen is significantly modified by the pH 6 level of acidity (effect $A_2 \times S$). There was no significant difference between observations within Cook and Rowden varieties; and nutritional effects introduced no significant modification in this comparison. Disease development in the Half & Half variety was markedly different

from that for the other two varieties, and most nutritional factors significantly modified this comparison.

There was a definite differential in the amount of wilt that developed up to the time of the first count, and that which developed during the second 9-day interval (effect T_1). This differential, due to time of observation, was conditioned by the level of nitrogen supply, the acidity of the substrate, and the source of nitrogen at the pH 6 level of acidity. Relationships involving the comparison between Cook and Rowden varieties were not significantly different at these two times of observation. Yet, comparisons between Half & Half and these other two varieties were distinct at these two times of observation. Furthermore, sources of N, levels of N, and S · L interaction definitely modified the disease comparisons within this $T_1 \cdot V_2$ effect.

That disease development was not linear over the period of observation is shown by the significance for T_2, the curvilinear or quadratic effect of the time factor. Furthermore, both sources of nitrogen and levels of nitrogen introduced significant modifications in the departure of rate of disease development from linearity. The quadratic effect of time had no more influence upon Cook and Rowden comparisons than did the linear effect of this factor. Yet, the Half & Half comparison with the more resistant varieties was influenced to a highly significant degree by both components of the time factor; and, in both instances, the relationship was further modified by the level and source of nitrogen supplied.

More detailed pathological deductions might be drawn from the analysis, but the foregoing is presented merely to provide an insight into the possibilities of the use of the mechanism. The physiological relationships involved are definitely influenced by the time factor, and the relative magnitude of the influence of the time effect upon other effects and their interactions may be easily isolated; especially so, as a result of the segregation of multiple degree of freedom factors into their individual components.

EXPERIMENT C

Many of the complications encountered in the analysis of Experiment B would have been avoided had the contributing factors all been at two levels instead of some of them being at three levels, thereby avoiding the calculation of quadratic effects.

Experiment C provides an illustration of the relative simplicity in the analysis of a factorial experiment involving contributing factors at only two levels. It involved a study of the growth of two varieties of cotton plants in sand culture, with the nutrient solutions varied as to two levels of nitrogen supply, two levels of phosphorous supply, and two levels of potassium supply. That is, it was characterized as a $2 \times 2 \times 2 \times 2$ design. There were six replicates of each variety-treatment combination, but, as shown by Figure 3, each three of these replicates comprised a single tier of pots across the greenhouse bench. This divided the experiment into two main blocks, each one on a single bench. In order to allow for any further position effects, the highest order inter-

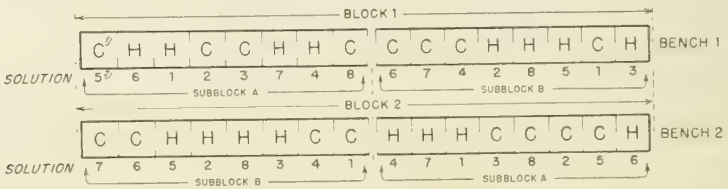
BLOCK 1
C H H C C H H C C C C H H H C H
BENCH 1
SOLUTION 5 6 1 2 3 7 4 8
6 7 4 2 8 5 1 3
SUBBLOCK A
SUBBLOCK B
BLOCK 2
C C H H H H C C H H H C C C C H
BENCH 2
SOLUTION 7 6 5 2 8 3 4 1
4 7 1 3 8 2 5 6
SUBBLOCK B
SUBBLOCK A

Figure 3. ARRANGEMENT OF CULTURES IN EXPERIMENT C.

[1] Key to varieties (each letter designates three jars):
C = Cook 307
H = Half & Half

[2] Key to treatments:
1 = Low N, low P, low K
2 = Low N, low P, high K
3 = Low N, high P, low K
4 = Low N, high P, high K
5 = High N, low P, low K
6 = High N, low P, high K
7 = High N, high P, low K
8 = High N, high P, high K

action was confounded with subblocks within the main blocks. The highest order interaction in a factorial experiment is seldom a significant contributor, and it is often advantageous to sacrifice whatever contribution may be made by this interaction in order to gain more information on location effects. The confounding of interactions, which may be expected to be of dubious significance, with subblock differences may be a valuable method in greenhouse experiments in which location effects may possibly be influential. In experiments involving several blocks, it is possible to confound different interactions within different blocks, a procedure known as "partial confounding." It has the advantage of permitting information to be gained on an interaction confounded in one block from the blocks in which it is unconfounded. Yates *(10)* has presented a rather extensive discussion of this procedure, but clarity in the present discussion may be

aided by reviewing a few of the elementary principles in the technique of confounding. In the experimental example used, only two main blocks were involved, and so only the interaction, $V \times N \times P \times K$, was confounded with subblocks.

In Experiment B it was shown that an algebraic formulation could be set up for the components of a given main effect or interaction. The $V \times N \times P \times K$ interaction would be formulated as follows:

$$V \times N \times P \times K = (v_1 - v_2)\ (n_1 - n_2)\ (p_1 - p_2)\ (k_1 - k_2)$$

which, on expansion, becomes

$$\begin{aligned} V \times N \times P \times K = {} & v_1 n_1 p_1 k_1 + v_1 n_1 p_2 k_2 + v_1 n_2 p_1 k_2 + v_1 n_2 p_2 k_1 + \\ & v_2 n_1 p_1 k_2 + v_2 n_1 p_2 k_1 + v_2 n_2 p_1 k_1 + v_2 n_2 p_2 k_2 - \\ & v_1 n_1 p_1 k_2 - v_1 n_1 p_2 k_1 - v_1 n_2 p_1 k_1 - v_1 n_2 p_2 k_2 - \\ & v_2 n_1 p_1 k_1 - v_2 n_1 p_2 k_2 - v_2 n_2 p_1 k_2 - v_2 n_2 p_2 k_1 \end{aligned}$$

In any 2^n experiment (a number of factors each of two levels) when a given interaction is to be confounded with subblocks, the treatments designated by the positive components of the expanded equation are placed in one subblock, and those designated by the negative components in the other subblock. Thus, in this experiment, wherein interaction $V \times N \times P \times K$ was confounded with subblocks, the treatments comprising the subblocks are as follows:

Subblock A	*Subblock B*
$v_1 n_1 p_1 k_1$	$v_1 n_1 p_1 k_2$
$v_1 n_1 p_2 k_2$	$v_1 n_1 p_2 k_1$
$v_1 n_2 p_1 k_2$	$v_1 n_2 p_1 k_1$
$v_1 n_2 p_2 k_1$	$v_1 n_2 p_2 k_2$
$v_2 n_1 p_1 k_2$	$v_2 n_1 p_1 k_1$
$v_2 n_1 p_2 k_1$	$v_2 n_1 p_2 k_2$
$v_2 n_2 p_1 k_1$	$v_2 n_2 p_1 k_2$
$v_2 n_2 p_2 k_2$	$v_2 n_2 p_2 k_1$

(v_1 = Half & Half; v_2 = Cook 307.)

Reference to Figure 3 will show each group of treatment combinations was randomized within the designated subblock.

The raw data obtained in this experiment for green weights of plants per culture are presented in Table 19. Although there are some obvious differences in the data of this table, the summaries of these data presented in Table 20 facilitate a consideration of the main contributors. One can readily deduce from these summaries that there was no difference in the mean growth response in terms of green weight between the two varieties. It is also evident that the various nutritional factors induced re-

TABLE 19. GREEN WEIGHTS, IN GRAMS, OF PLANTS PER CULTURE AT TIME OF HARVEST

Variety	Solution No.	Block I			Block II		
		Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
		Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Cook 307	1	55	62	58	46	46	47
Cook 307	2	55	65	59	49	52	46
Cook 307	3	75	75	78	69	71	73
Cook 307	4	86	74	79	89	82	75
Cook 307	5	53	54	52	60	52	50
Cook 307	6	48	63	57	66	44	40
Cook 307	7	145	143	137	136	145	126
Cook 307	8	291	270	280	245	230	260
Half & Half	1	55	55	55	51	43	51
Half & Half	2	56	52	47	41	43	46
Half & Half	3	73	84	78	70	57	63
Half & Half	4	86	76	75	81	80	71
Half & Half	5	52	54	49	40	40	43
Half & Half	6	63	61	52	58	66	58
Half & Half	7	171	156	158	144	114	110
Half & Half	8	262	253	250	270	250	295

markably uniform growth response for the two varieties of plants, indicating a definite lack of significance for variety × nutrient interactions. However, it is apparent that each of the three nutritional factors had a marked influence on growth response, and that it is possible that interactions between these main factors are making a considerable contribution to total variability in the experiment. In order to arrive at a measure of the importance of the latter as well as all other induced variables, Yates' *(10)* tabular method may be used. This method is particularly adapted to a 2^n experiment. It also is a particularly advantageous method when mechanical calculators are not available.

The mechanics for the calculation of the main effects and interactions of Experiment C are presented in Table 21. Under "green weights" are the sums of the observations for the six replicates in a given treatment. These figures must be entered into the table in definite order. The first entry must be that for the treatment which consists of the low levels of all contributors (Half & Half is arbitrarily chosen to be the low level of the variety factor). The capital letter designations under "variance contributor" then not only designate main effects and interactions found under "effects," but also show which factors are at the high level in the treatments associated with a given growth response. That is, "variance contributor O" also designates the treatment with all factors at low levels; "N," the treatment with all factors but N at low levels; "N · P," the treatment with all factors

except N and P at low levels; etc. The factors are introduced into the table in a "standard order" of the type shown. As each factor is introduced in turn, it is followed by all combinations of itself and the factors previously introduced.

After setting up the summations for each treatment under "green weight," the formation of column "A" is the next step. The first eight numbers in column "A" are the sums of the eight successive pairs of numbers in the "green weights" column, and the second eight numbers are the differences between these eight successive pairs. For example, $310 + 278 = 588$ and $310 + 278 = -32$. Column "B" is formed from column "A" in the same manner, "C" is formed from "B," and "D" is formed from "C." The total (9,171) and the main effect and interaction totals are given in column "D." The only check on the accuracy of the manipulations is an independently derived value for the total at the head of column "D," and, consequently, absolute accuracy is necessary in the mechanics of developing this table, with particular attention being given to signs. This method is essentially the same as that illustrated by Table 13 in the discussion of Experiment B.

TABLE 20. MEAN VALUES FOR ALL MAIN EFFECTS AND FIRST ORDER INTERACTIONS OF DATA IN TABLE 19

Variety	Low N	High N	Low P	High P	Low K	High K	Mean
Cook 307	65.2	126.9	53.3	138.6	79.5	112.7	96.1
Half & Half	62.2	127.9	51.3	138.7	77.7	112.2	94.9
Mean	63.7	127.4	52.3	138.8	78.6	112.4	

N level	Low P	High P	Low K	High K
Low N	51.4	75.8	62.1	65.2
High N	53.1	201.7	95.2	159.6
Mean	52.3	138.7	78.6	112.4

P level	Low K	High K
Low P	51.0	53.6
High P	106.3	171.2
Mean	78.7	112.4

TABLE 21. CALCULATION OF MAIN EFFECTS AND INTERACTIONS OF EXPERIMENT C

Variance contributor	Green weight	Effect			
		A	B	C	D
	Grams				
O	310	588	1,866	4,558	9,171
N	278	1,278	2,692	4,613	3,061
P	425	643	1,908	1,580	4,151
N × P	853	2,049	2,705	1,481	2,981
K	285	635	396	2,096	1,623
N × K	358	1,273	1,184	2,055	1,473
P × K	469	644	398	1,498	1,495
N × P × K	1,580	2,061	1,083	1,483	1,293
V	314	— 32	690	826	55
N × V	321	428	1,406	797	— 99
P × V	441	73	638	788	— 41
N × P × V	832	1,111	1,417	685	— 15
K × V	326	7	460	716	— 29
N × K × V	318	391	1,038	779	—103
P × K × V	485	— 8	384	578	63
N × P × K × V	1,576	1,091	1,099	715	137

The analysis of variance of these data is given in Table 22. The values for total sums of squares and for main block and total block sums of squares are calculated according to the usual procedure by the machine method. The sums of squares for each effect and interaction are calculated by squaring the respective values in the "D" column and dividing by 96 (the total number of observations). Hence,

$$\text{N effect} = \frac{(3{,}061)^2}{96} = 97{,}601.3$$

In setting up the analysis in Table 22, it is to be noted that since interaction N × P × K × V was confounded with subblocks, it must be included in the three degrees of freedom for total block variance. This latter value was highly significant, but it is evident that this significant difference between blocks was entirely due to difference in plant response on the two benches (main block comparison).

The deduction drawn from Table 20, that variety comparisons were of no significance and that nutrient main effects were definitely significant, is well established by the analysis. In fact, it is rather outstanding that all interactions between nutrient effects are very highly significant in accounting for variability in the data. The variance assignable to associations involving V is practically nil by comparison. However, the variance associated with N × P × K × V interaction is distinctly larger than for any other comparison involving V, which is the reverse of

that usually found for the highest order interaction. Hence, the inference may be readily drawn that the variance associated with interaction N × P × K × V is in reality due to the placement effects between subblocks with which this interaction is confounded.

It was mentioned in the discussions of Experiments A and B as well as in this one that in order to avoid errors of nutrient application, a given nutrient treatment was applied to each of three pots across the bench comprising a tier or split-plot; and it may be logically argued that the tier of three pots should be taken as a unit rather than the individual pots. In the latter case the total number of observations would be 32 instead of 96, resulting in a loss of 64 degrees of freedom for error variance. Table 23 presents the analysis of these data according to tiers or split-plots. According to these results, total block variance is not significant, and the difference between benches (main blocks) attains only the 5 per cent level of significance. Significance for all other contributors is identical by both methods. Although some of the loss in significance for block effects by the split-plot method may be due to the loss in degrees of freedom for error variance, the latter method is productive of variance ratios of much smaller value. As far as interpretations of induced effects are concerned, it makes no difference which of these two analyses

TABLE 22. ANALYSIS OF VARIANCE OF TABLE 18

Source of variation	Sum of squares	D.F.	Mean square	F value
Total	470,865.9	95		
Blocks: Between main blocks	1,692.8	1	1,692.8	16.56**
Blocks: N × P × K × V	195.5	1	195.5	1.91
Blocks: Within block pairs	296.5	1	296.5	2.90
Blocks: Total	2,184.8	3	728.3	7.13**
Treatments—				
N	97,601.3	1	97,601.3	955.00**
P	179,487.5	1	179,487.5	1,756.24**
N × P	92,566.3	1	92,566.3	905.74**
K	27,438.8	1	27,438.8	268.48**
N × K	22,601.3	1	22,601.3	201.58**
P × K	23,281.5	1	23,281.5	227.80**
N × P × K	17,415.1	1	17,415.1	170.40**
V	31.5	1	31.5	
N × V	102.1	1	102.1	
P × V	17.5	1	17.5	
N × P × V	2.3	1	2.3	
K × V	8.8	1	8.8	
N × K × V	110.5	1	110.5	1.08
P × K × V	41.3	1	41.3	
Discrepance	7,975.3	78	102.2	

** High significance.

TABLE 23. ANALYSIS OF VARIANCE OF TABLE 18 TAKING INTO ACCOUNT SPLIT-PLOTS

Source of variation	Sum of squares	D.F.	Mean square	F value
Total	1,302,704.0	31		
Blocks: Between main blocks	5,075.3	1	5,075.3	6.21*
Blocks: N × P × K × V	586.5	1	586.5	
Blocks: Within block pairs	892.8	1	892.8	1.09
Total	6,554.6	3	2,184.9	2.67
Treatments—				
N	292,803.8	1	292,803.8	358.39**
P	538,462.5	1	538,462.5	659.07**
N × P	277,698.8	1	277,698.8	339.90**
K	82,316.5	1	82,316.5	100.75**
N × K	67,804.0	1	67,804.0	82.99**
P × K	69,844.5	1	69,844.5	85.49**
N × P × K	52,245.3	1	52,245.3	63.95**
V	94.5	1	94.5	
N × V	306.3	1	306.3	
P × V	52.5	1	52.5	
N × P × V	7.0	1	7.0	
K × V	26.3	1	26.3	
N × K × V	331.5	1	331.5	
P × K × V	124.0	1	124.0	
Discrepance	11,437.9	14	817.0	

* Low significance. ** High significance.

is followed, but strict compliance with the implications of the design of the experiment requires the procedure of Table 23. It will rest with each experimenter to decide whether to use a design that will simplify manipulation but forfeit precision, or to follow complete randomization and aim for highest accuracy in coping with the associated complications in handling the mechanics of the experiment.

EXPERIMENT D

The simplicity of confounding a 2^n experiment, as shown in the preceding section, does not hold for one in which one or more of the factors is studied at three levels. In fact, designs involving factors at both two and three levels may introduce appreciable complications, especially if one wishes to confound a specific interaction with subblocks. Experiment D illustrates the treatment of a $2 \times 2 \times 2 \times 3$ design, the arrangement of the individual pots being shown in Figure 4. Two varieties of cotton plants were grown in sand culture at two levels of nitrogen supply, with two sources of nitrogen, and at three pH levels. Each treatment-variety combination was replicated four times, with two replicates comprising a split-plot across the bench. The two main blocks, which extended laterally across the three benches, accounted for the other two replicates. Each of the main blocks was divided

by confounding the third-order interaction V · S · L · A with subblocks. The treatment-variety combinations were randomized within subblocks.

The question may be raised as to why the block arrangement chosen extended laterally across the benches instead of being confined to benches. The necessity for this may be brought out by a consideration of the problem of confounding in this experiment. It will be seen from Figure 4 that each subblock contains 12 split-plots or duplicate jars of specified variety-treatment combinations. To be sure, each of the 12 treatments occurs once and only once in each subblock, and each variety occurs six times in each subblock. Since there are 24 variety-treatment combinations in this experiment, it is possible to confound interaction V × S × L × A, involving the four factors ($2 \cdot 2 \cdot 2 \times 3 = 24$) between two subblocks of 12 plots each. This is shown by Table 24; *e. g.*, all nutrient treatments occur once in each subblock, and varieties six times each, but any treatment occurring with one variety in one subblock occurs with the other variety in the other subblock. Hence, all nutrient effects and interactions are unconfounded, a contingency made possible by the fact that the products of the factors for each of these main effects and interactions is a factor of 12 (the number of plots in a subblock). Thus, for S × L, $2 \times 2 = 4$, which is a factor of 12; for S · A, $2 \times 3 = 6$, which also is a factor of 12; for S · L · A, $2 \times 2 \cdot 3 = 12$, another factor of 12; etc. Similarly, it may be shown that most inter-

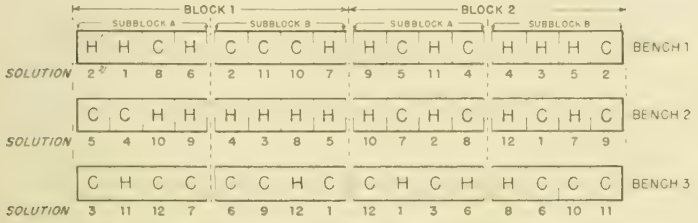
BLOCK 1
BLOCK 2
SUBBLOCK A
SUBBLOCK B
SUBBLOCK A
SUBBLOCK B
H H C H C C C H H C H C H H H C BENCH 1
SOLUTION 2 2 1 8 6 2 11 10 7 9 5 11 4 4 3 5 2
C C H H H H H H H C H C H C H C BENCH 2
SOLUTION 5 4 10 9 4 3 8 5 10 7 2 8 12 1 7 9
C H C C C C H C C H C H H C C C BENCH 3
SOLUTION 3 11 12 7 6 9 12 1 12 1 3 6 8 6 10 11

Figure 4. ARRANGEMENT OF CULTURES IN EXPERIMENT D.

[1] Key to varieties (each letter designates two jars):
C = Cook 307
H = Half & Half

[2] Key to treatments:
1 = Low nitrate, pH 4
2 = Low nitrate, pH 6
3 = Low nitrate, pH 8
4 = High nitrate, pH 4
5 = High nitrate, pH 6
6 = High nitrate, pH 8
7 = Low ammonium, pH 4
8 = Low ammonium, pH 6
9 = Low ammonium, pH 8
10 = High ammonium, pH 4
11 = High ammonium, pH 6
12 = High ammonium, pH 8

TABLE 24. MANNER OF CONFOUNDING EXPERIMENT D

Solution No.	Variety	Source of N	Level of N	pH (Acidity)
		SUBBLOCK A		
1	Half & Half	Nitrate	Low	Low
2	Half & Half	Nitrate	Low	Medium
3	Cook 307	Nitrate	Low	High
4	Cook 307	Nitrate	High	Low
5	Cook 307	Nitrate	High	Medium
6	Half & Half	Nitrate	High	High
7	Cook 307	Ammonium	Low	Low
8	Cook 307	Ammonium	Low	Medium
9	Half & Half	Ammonium	Low	High
10	Half & Half	Ammonium	High	Low
11	Half & Half	Ammonium	High	Medium
12	Cook 307	Ammonium	High	High
		SUBBLOCK B		
1	Cook 307	Nitrate	Low	Low
2	Cook 307	Nitrate	Low	Medium
3	Half & Half	Nitrate	Low	High
4	Half & Half	Nitrate	High	Low
5	Half & Half	Nitrate	High	Medium
6	Cook 307	Nitrate	High	High
7	Half & Half	Ammonium	Low	Low
8	Half & Half	Ammonium	Low	Medium
9	Cook 307	Ammonium	Low	High
10	Cook 307	Ammonium	High	Low
11	Cook 307	Ammonium	High	Medium
12	Half & Half	Ammonium	High	High

actions involving the variety comparison may be wholly unconfounded. Interaction V × S × L is an exception to this since 2 × 2 × 2 or 8 is not a factor of 12, and, hence, such an interaction may not be orthogonally unconfounded with subblocks of 12 plots. In other words, there are two split-plots of Half & Half receiving low nitrate nitrogen supply in subblock A, whereas there is only one of Cook receiving this nutrient combination. The reciprocal situation is present in subblock B. A similar inequality exists for the distribution of the other level-source-variety combinations within the respective subblocks. However, this interaction, V × S × L, is the only one that lacks orthogonality with subblocks in this design.

Regarding the supposition that it might have been preferable to form three subblocks within each main block and thereby permit one-half of each bench to be a composite subblock, it must be remembered that such a setup would permit only eight plots per subblock. In such an arrangement, effect A and interactions S × A, L × A, V × A, S × L × A, S × V × A, L × V × A, and S × L × V × A would neither be wholly unconfounded with subblocks nor would they be orthogonally confounded with subblocks,

since none of the numerical factors associated with these effects and interactions are factors of the number, 8. It would be possible to gain information on effects the confounding of which is not orthogonal with subblocks, but the procedure is involved and cumbersome *(10)*.

Table 25 presents the data for the green weights of plants per culture at the time of harvest. Since each pair of cultures in a tier on the bench comprised a split-plot, the summation of each respective pair of green weight observations is also given, in view of the fact that these summation figures are the ones to be used in the analysis of variance. But before proceeding to the analysis, consideration should be given to the mean values for the main effects and first order interactions given in Table 26. From this table, it appears that sources of nitrogen, *per se*, induced no differential in growth response, that levels of nitrogen and varieties induced a dubious differential, and that acidity levels were associated with growth response that may be significantly different, which would lead one to believe that there were no significant interactive effects between the variety comparison and nutrient contributors. Further, there is no indication that the interactions between levels of nitrogen and either sources or pH

TABLE 25. GROWTH OF PLANTS IN EXPERIMENT D IN TERMS OF GREEN WEIGHT PER POT

Variety	Solution No.	Block I			Block II		
		No. 1	No. 2	(1 + 2)	No. 1	No. 2	(1 + 2)
		Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Cook 307	1	245	260	505	255	230	485
Cook 307	2	240	225	465	240	240	480
Cook 307	3	170	165	335	170	180	350
Cook 307	4	305	355	660	245	265	510
Cook [illegible]	5	295	265	560	250	280	530
Cook [illegible]	6	135	190	325	155	160	315
Cook [illegible]	7	210	220	430	200	220	420
Cook [illegible]	8	235	225	460	240	240	480
Cook [illegible]	9	225	240	465	235	220	455
Cook [illegible]	10	210	215	425	185	215	400
Cook [illegible]	11	200	230	430	235	280	515
Cook [illegible]	12	310	300	610	265	240	505
Ha[illegible] & Half	1	240	235	475	250	245	495
Ha[illegible] & Half	2	220	235	455	230	235	465
Ha[illegible] & Half	3	150	145	295	155	135	290
Ha[illegible] & Half	4	295	285	580	240	250	490
Ha[illegible] & Half	5	285	300	585	270	270	540
Ha[illegible] & Half	6	135	160	295	160	150	310
Ha[illegible] & Half	7	170	195	365	210	195	405
Ha[illegible] & Half	8	230	210	440	200	240	440
Ha[illegible] & Half	9	215	225	440	200	240	440
Ha[illegible] & Half	10	170	200	370	160	200	360
Ha[illegible] & Half	11	265	245	510	200	180	380
Ha[illegible] & Half	12	270	230	500	260	235	495

levels are significant. There does appear, however, to be a definite interrelationship between sources of nitrogen and pH levels in their effect upon growth response. The experiment is particularly interesting in that so few of the main contributors could be considered definitely significant by inspection. This is in strong contrast with the situation found in Experiment C, wherein the significance of the main effects and first order interactions were so evident by inspection of the summarized data that the value of the analysis was definitely diminished. In Experiment D an analysis is essential before any conclusions may be drawn.

Table 27 presents the summated yields in grams of green weight for the four replicates in each variety-treatment combination, together with the calculated effect columns. Each of the three columns of calculated effects for pH levels were calculated from the respective yield column by the method used for setting up Table 21 (Experiment C). The intermediate steps are omitted, but the inclusion of a three-level factor necessitates a modification in this tabular method — that of obtaining the sums and differences within each level of the three-level factor independently. Under

TABLE 26. MEAN VALUES FOR MAIN EFFECTS AND FIRST ORDER INTERACTIONS OF DATA IN EXPERIMENT D

Variety	NO_3 N	NH_4 N	Low N	High N	pH 4	pH 6	pH 8	Mean
Cook 307	230.2	233.5	222.1	241.5	239.5	248.1	210.0	232.6
Hall & Hall	219.8	211.6	205.8	225.6	217.8	239.7	189.7	215.8
Mean	225.0	222.5	213.9	233.5	228.7	243.9	200.0	224.2

Treatment	Low N	High N	pH 4	pH 6	pH 8
NO_3	212.3	237.5	262.5	255.0	157.2
NH_4	215.6	229.6	194.9	230.2	242.5
Mean	213.9	233.5	228.7	242.7	199.8

Treatment	pH 4	pH 6	pH 8
Low N	220.3	231.6	190.0
High N	237.2	253.7	209.6
Mean	228.7	242.7	199.8

TABLE 27. YIELDS AND CALCULATED EFFECTS OF EXPERIMENT D

Source	Level	Variety	Yield			Net effects				Designation
			pH 4	pH 6	pH 8	pH 4	pH 6	pH 8	Sums	
Nit.	Low	H[1]	970	920	585	7,375	7,755	6,395	21,525	O
Am.	Low	H	770	900	850	—1,025	—405	1,365	— 65	S
Nit.	High	H	1,070	1,125	605	215	345	315	875	L
Am.	High	H	730	890	995	— 345	—355	365	—335	S × L
Nit.	Low	C[1]	990	945	685	295	85	325	705	V
Am.	Low	C	850	940	920	55	105	55	215	S × V
Nit.	High	C	1,170	1,090	640	95	— 45	— 15	35	L × V
Am.	High	C	825	945	1,115	— 65	75	115	125	S × L × V

[1] H = Half & Half ; C = Cook 307.

"sums" in Table 27 are present the algebraic sums of each set of three associated pH effects. The sums of squares for main effects and interactions involving only two-level factors is calculated directly from the "sums" column by squaring the net arithmetical effect and dividing by 48. Sums of squares for comparisons involving the three-level acidity factor are derived by dividing the sum of the squares of each of three associated acidity effects by 16 and subtracting the value for the respective sum of squares derived from the "sums" column. For example, sum of squares for A equals

$$\frac{(7{,}375)^2 + (7{,}755)^2 + (6{,}395)^2}{16} - \frac{(21{,}525)^2}{48}$$

and sum of squares for A × S equals

$$\frac{(-1{,}025)^2 + (-405)^2 + (1{,}365)^2}{16} - \frac{(-65)}{48}$$

The analysis of variance of the data in this experiment is presented in Table 28. None of the components of block effects were significant contributors to the variance. Due to the fact that interactions S × L × V and S × L × A × V were confounded with subblock differences, the variance for these interactions may not be included in the total variance from known sources. These two mean squares were included in the table to show that, even if dependence could be placed upon them, their contribution would be insignificant.

Certain unusual features in these data merit special comment. The mean square for the effect of sources of nitrogen did not even approach the level of significance, and yet, when sources were studied as to their interrelationship with acidity levels (interaction S × A), the relation was found to be associated with the largest mean square in the experiment. Although such

an observation is noteworthy from the statistical point of view, the student of nitrogen sources in plant nutrition work will regard such results as being logical. The high significance found for interaction S × L × A indicates that the level of nitrogen supply modifies the interrelationship between nitrogen sources and acidity levels. Also, there was a significant difference in the growth response of the two varieties in this experiment, whereas such a differential was absent in Experiment C. However, no nutritional effect induced a significant modification in the variety comparison. Statistical results similar to these indicate that such analyses may make a definite contribution to plant nutrition work by aiding in the evaluation of physiological relationships.

This experimental example gave an indication of the complexity inherent in the confounding of an experiment in which some factors are at two levels and some at three. Although bench space did not permit an ideal arrangement of the blocks, it is believed that the method of confounding used was the most desirable. Experiments involving more than one factor at three

TABLE 28. ANALYSIS OF VARIANCE OF EXPERIMENT D

Source of variation	Sum of squares	D.F.	Mean square	F value
Total	347,107.82	47		
Blocks: Between main blocks	4,700.52	1	4,700.52	3.52
Blocks: Between subblock pairs	229.69	1	229.69	
Blocks: Within block means	1,813.02	1	1,813.02	1.36
Total	6,743.23	3	2,247.74	1.69
Treatments—				
S	88.02	1	88.02	
L	15,950.52	1	15,950.52	11.96**
S × L	2,338.02	1	2,338.02	1.75
A	61,550.00	2	30,775.00	23.07**
S × A	192,279.17	2	96,139.58	72.08**
L × A	579.17	2	289.58	
S × L × A	21,304.17	2	10,652.08	7.99**
V	10,354.69	1	10,354.69	7.76*
S × V	963.02	1	963.02	
L × V	25.52	1	25.52	
S × L × V [1]	325.52	1	325.52	
A × V	2,137.50	2	1,068.75	
S × A × V	104.17	2	52.08	
L × A × V	679.17	2	339.58	
S × L × A × V [1]	1,116.67	2	558.33	
Error	32,011.45	24	1,333.81	

* Low significance.
** High significance.
[1] Confounded with subblocks, and, hence, eliminated from total treatment variance.

levels present further complexity. [For a technical discussion of such problems, see Yates *(10)*.]

SUMMARY

The design and analysis of four factorial plant nutrition experiments carried out in the greenhouse have been presented. The weaknesses in some of these designs have been pointed out, together with circumstances prevailing under certain greenhouse manipulations that tend to obviate the apparent inferiorities of the unorthodox arrangements. A satisfactory procedure for the analysis of variance is presented in each case with the inclusion of a few possible modifications.

Experiment A is a $3\times 3\times 2\times 2$ design with four replicates comprising four blocks. Main effects were confounded with split-plots, and the procedure was presented for correctly taking into account such a contingency, and for segregating the components of a two-degree-of-freedom factor into its individual components. A method applicable for studying plant disease data of the type presented on a proportionate basis was suggested.

Experiment B presents a $3\times 3\times 3\times 2\times 2$ design with time of observation considered as one of the factors. A rather detailed study of the proper method of taking into account the time factor is included. The method of analyzing the data, according to single degrees of freedom with individual error terms, is presented. A comparison of results obtained by using the individual error terms, the fully pooled error, and grouped errors provides a basis for discussing the relative merits of various methods of treatment of error.

Experiment C illustrates a $2\times 2\times 2\times 2$ arrangement. It demonstrates the simplicity of the analysis by the tabular method possible in a 2^n design. The principle contribution of the description of the experiment was in the illustration of the advisability of confounding a higher order interaction with subblocks, together with the procedure involved.

Experiment D demonstrates the technique of confounding a $3\times 2\times 2\times 2$ experiment, together with its analysis by the tabular method. The presence of a three-level factor appreciably complicates the procedure, but the method presented has been found to be satisfactory.

Nò attempt has been made in this presentation to explain the physiological or pathological importance of the results of the four experiments. This phase of the work will be presented later.

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